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ON THE TISSUE ALDEHYDE SHIFT IN THE RAT KIDNEY

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IT HAS BEEN SHOWN by Oster (1945) that the tissue aldehydes in the intercortico-medullary zone (ICM zone) of the female rat kidney undergo changes synchronized with the estrous cycle. The aldehydes tend to decrease in the diestrous phase of the cycle and during pregnancy, and they increase during the estrous phase.

The present study was undertaken to investigate the effect of the principally known female and male sex hormones, progesterone, estrogens and testosterone, on this phenomenon. In addition, the influence of diethylstilbestrol, desoxycorticosterone acetate and pregnenolone were studied.

METHODS

Fuchsin sulfurous acid stains on frozen sections of unfixed kidneys were made in the same manner as previously described (Oster and Mulinos, 1944). Albino rats of various strains were used for this investigation. Progesterone, testosterone propionate and α-estradiol benzoate were dissolved in peanut oil so that 0.3 cc. solution contained the daily dose. Peanut oil was given as control. All injections were intramuscular. Diethylstilbestrol, 1 mg. in 0.1 cc. peanut oil, was given by mouth. The animals were maintained on a normal mixed diet enriched with B complex vitamins.

RESULTS

Before studying the influence of any substance on the aldehyde content of the ICM zone one must first be certain that the zone has a

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constant intensity of staining when treated with the reagents. Previously described injections (Oster, 1945) into mature female rats proved inconclusive, because it could not be demonstrated that eventual changes were due to the injections rather than to normal physiological processes.

Suitable test animals showing reliable staining of the ICM zone were found in the normal male rat and in the female rat ovariectomized before puberty and then permitted to grow. The females were ovariectomized when they weighed 60 to 70 grams. Only animals with immature ovaries and closed vaginas were used. The kidneys of 15 such operated animals showed deeply stained ICM zones from 7 to 117 days postperatively. These animals were deemed suitable for testing substances which might cause a disappearance of aldehydes from the ICM zone.

Ovariectomized Animals

Results of either injecting or feeding forty animals ovariectomized before puberty are recorded in Table 1.

The evaluation of the staining quality of tissue aldehydes in the

TABLE I. ALDEHYDE STAINING PATTERNS IN THE ICM ZONES OF TREATED AND UNTREATED OVARIECTOMIZED RATS

No. of rats used	Days post-ovariectomy	Substance given	Dose	No. of days administered	Staining of ICM zone	Remarks[1]
15	7–117				+ +	
2	78	α-estradiol benzoate	50 μg.	5	(+) (+)	
1	61	α-estradiol benzoate	50 μg.	8	− (+)	
1	89	α-estradiol benzoate	50 μg.	11	(−) (−)	
1	57	α-estradiol benzoate	50 μg.	11	− −	
1	108	diethylstilbestrol	1 mg.	4	(−) +	
1	43	diethylstilbestrol	1 mg.	10	− (−)	
4	16	diethylstilbestrol	1 mg.	10	− −	
2	74	progesterone	3 mg.	5	+ +	
2	57	progesterone	2 mg.	5	(+) (+)	P
1	82	progesterone	2 mg.	10	(+) (+)	P
1	42	progesterone	2 mg.	10	− (+)	
1	25	progesterone	3 mg.	10	− −	
1	25	progesterone	3 mg.	10	(−) (−)	
2	125	pregnenolone	3 mg.	5	(+) (+)	P
2	35	pregnenolone	3 mg.	10	(+) (+)	P
2	42	pregnenolone	3 mg.	10	+ +	
2	66	desoxycorticosterone acetate	3 mg.	5	(+) (+)	P
1	78	desoxycorticosterone acetate	3 mg.	10	(+) (+)	P
1	89	desoxycorticosterone acetate	3 mg.	10	− −	P
2	42	desoxycorticosterone acetate	3 mg.	10	+ +	
1	31	desoxycorticosterone acetate	3 mg.	10	− +	
2	25	testosterone	3 mg.	10	+ +	
1	38	testosterone	3 mg.	10	+ +	
3	57	testosterone	3 mg.	11	+ +	
2	56	peanut oil	0.3 cc.	10	+ +	

[1] Animals marked "P" were primed with three previous injections of 50 μg. α-estradiol benzoate. A vaginal smear after the third day showed extensive cornification of the epithelial cells.

kidney is not completely objective. However, the changes in the aldehyde reaction are so distinct as to be observed with the naked eye. To obtain comparable results, the grade scale which was used in a previous publication (Oster, 1945) was again utilized, and more definite criteria for the various symbols were set.

Staining intensity of the ICM zone is expressed by two symbols, the first referring to the lower half of the zone adjacent to the area of Henle's loops, and the second referring to the upper half of the zone bordering on the kidney cortex. Thus, ++ denotes an ICM zone intensively stained throughout. (+) (+) signifies destaining in such a way that the color intensity is similar to that found in the area of Henle's loops. In the case of the (+)+ reaction, a separation of the two zones is difficult, and the ICM zone appears narrowed to half its usual size. (−)(−) or —— indicates an ICM zone definitely less stained than Henle's loops region. Thus, a −+ ICM zone contains a completely destained lower portion and an intensively stained upper portion, giving the appearance of a pale halo surrounding the area of Henle's loops.

Evaluation of the findings in Table I shows that all the animals treated with α-estradiol benzoate or with diethylstilbestrol show a definite reduction of tissue aldehydes in the ICM zone. The effects of progesterone, pregnenolone and desoxycorticosterone acetate were not uniform. In some cases they had no effect for the given time and dose, and in other cases they decreased the aldehyde content of the ICM zone. Testosterone seemed to enlarge the ICM zone at the expense of the cortex. The peanut oil as control had no effect. Priming of the animals with α-estradiol benzoate for three days and subsequent injection of progesterone, pregnenolone or desoxycorticosterone acetate diminished uniformly the staining intensity of the ICM zone.

Male Animals

Fifteen normal mature male rats weighing from 130 to 200 grams were either injected with various steroids or fed diethylstilbestrol. Table 2 shows the results of the ICM zones of these animals. A ten day treatment period had almost no effect, but a twenty day period of either injecting α-estradiol benzoate or feeding diethylstilbestrol resulted in a complete disappearance of the tissue aldehydes of the ICM zone. This was the first time that the phenomenon was noted in male rats. Progesterone had a destaining effect after twenty days injection.

Pregnant Rats

The ICM zones of ten pregnant rats were examined either shortly before, at, or shortly after parturition. The results are summarized in Table 3.

With the exception of one animal, all pregnant rats showed a decrease in the staining of the ICM zone ranging from −+ to −−. Two

TABLE 2. THE INFLUENCE OF FEMALE SEX HORMONES ON THE ALDEHYDE REACTION IN THE ICM ZONES OF NORMAL MALE RATS

No. of rats used	Substance given	Dose	No. of days administered	Staining of ICM zone	
20				+	+
2	α-estradiol benzoate	50 μg.	11	(+)	(+)
2	α-estradiol benzoate	50 μg.	20	−	−
1	diethylstilbestrol	1 mg.	9	+	+
2	diethylstilbestrol	1 mg.	21	−	−
1	diethylstilbestrol	1 mg.	21	−	(−)
1	diethylstilbestrol	1 mg.	21	(+)	(+)
1	progesterone	3 mg.	10	(+)	+
1	progesterone	3 mg.	10	+	+
3	progesterone	3 mg.	20	(−)	+
1	progesterone	3 mg.	26	(−)	+

animals were bilaterally ovariectomized and unilaterally nephrectomized in one operation. Twenty three days later they were sacrificed and the remaining kidneys observed. The previously unstained ICM zone of one animal remained unchanged. The other showed an increase in staining intensity from − − to− (+). Bilateral ovariectomies were performed on two pregnant rats four and two days ante partum. The kidneys were examined 34 days and 26 days respectively, when uni-

TABLE 3. ALDEHYDE REACTION IN THE ICM ZONES OF PREGNANT RATS

No. of rats used	Examined Days	a.p.[1] or p.p.[1]	Staining of ICM zone		Remarks
2	2	a.p.	−	(+)	
1	2	a.p.	−	−	
1	0	p.p.	(+)	+	
1	0	p.p.	−	−	Left Nephrectomy and Bilateral Ovariectomy
	29	p.p.	−	(−)	Right Kidney
1	2	p.p.	−	+	
1	2	p.p.	−	+	Left Nephrectomy and Bilateral Ovariectomy
	23	p.p.	−	+	Right Kidney
1	3	p.p.	−	(+)	
1	3	p.p.	−	−	Left Nephrectomy and Bilateral Ovariectomy
	23	p.p.	−	(+)	Right Kidney
1	6	p.p.	−	+	
	4	a.p.			Bilateral Ovariectomy
1	34	p.p.	−	+	Left Nephrectomy
	55	p.p.	+	+	Right Kidney
	2	a.p.			Bilateral Ovariectomy
1	26	p.p.	(+)	+	Left Nephrectomy
	66	p.p.	+	+	Right Kidney

[1] a.p. ante partum, p.p. post partum.

lateral nephrectomies were performed, and 55 days and 66 days post partum respectively, when the animals were sacrificed. In each case the ICM zone showed a tendency to increase its staining quality in spite of removal of the ovaries.

Duration of the Destained State of the ICM Zone after Treatment

Eight ovariectomized animals were unilaterally nephrectomized after treatment with the various aldehyde-diminishing steroids. The remaining kidneys were examined 17 days later in six animals and 31 days later in two animals. In most cases there was a slight but noticeable tendency for the ICM zone to revert to its original deeply stainable state. However, one animal retained, even after 31 days, the same lack of stainability in the ICM zone as was noticed in the previously examined kidney.

Effect of Testosterone

Six ovariectomized animals were given daily oral feedings of 1 mg. diethylstilbestrol for ten days. At this time their left kidneys were removed and the ICM zones found to be completely unstainable. Three of these animals were then given intramuscular injections of 3 mg. testosterone propionate daily for ten consecutive days. The other three animals remained untreated and served as controls. In all three rats which had received testosterone the ICM zones were found to be ++. In the control group th ICM zones were still completely unstainable.

On the other hand, four animals which had received ten daily injections of testosterone failed to show a decrease in the staining reaction in the ICM zones after a subsequent ten day treatment with 1 mg. diethylstilbestrol daily by mouth. Five animals shown in Table 1 which received the same amount of diethylstilbestrol during the same period of time showed a definite decrease in stainability in their ICM zones. Testosterone, therefore, seems to influence the metabolism of tissue aldehydes in such a manner that either tissue aldehydes are deposited or depletion of stored aldehydes is prevented.

Vitamin Deficient Animals

Male rats seriously deficient in vitamin A, vitamin D and the B complex vitamins were found to have the normal staining reaction in the ICM zone of the kidney.

CHEMICAL STUDIES

Preliminary chemical extractions were performed in order to obtain information about the nature of the aldehydes present in kidney tissue. Because of the small size of the rat kidney and the greater availability of hog kidney, the latter organs were used for the study.

It is understood that the results gained in this experiment cannot be fully translated into the properties of rat tissue aldehydes.

By suitable extraction with ethyl acetate after acid hydrolysis, the bisulfite addition products of tissue aldehydes were obtained. After careful hydrolysis and steam distillation, two types of aldehydes were observed. The major fraction was insoluble in 20% alcohol and was proven, by mixed melting point determination after silver oxide oxidation, to be stearic acid. Palmital was not found in the hog kidney. The lesser fraction of the aldehydes was alcohol soluble. A dinitrophenylhydrazone derivative could be obtained from it. However, estimation of the molecular weight by nitrogen determination gave extremely high values which might be ascribed to polymerization. Feulgen (1929) also thought that aldehydes other than palmital and stearal were present in tissues. He suggested that they might be those of the unsaturated fatty acids. Oster (1942) suggested that aromatic aldehydes, which may be stored as tissue aldehydes, are formed in the process of amino acid metabolism.[1]

STUDIES ON MICE

Mice, like rats, have deeply stainable ICM zones in the kidneys. However, they exhibit no aldehyde shift phenomenon, as was found in the study of ten female animals in various phases of the estrous cycle. Five pregnant mice showed no failure of staining in the ICM zone. Bilateral ovariectomy or simultaneous bilateral ovariectomy and adrenalectomy were likewise without any effect on the staining intensity of the ICM zone. Feeding of 0.5 mg. of diethylstilbestrol in 0.05 cc. of peanut oil to ovariectomized animals up to 36 days did not change the ICM zone in the five animals studied. Feeding of 0.5 mg. diethylstilbestrol for seven days to an ovariectomized and adrenalectomized mouse still left the stainability of the ICM zone intact.

DISCUSSION OF RESULTS

From the present findings it may be concluded that α-estradiol benzoate, progesterone, diethylstilbestrol and desoxycorticosterone acetate effect a depletion of the tissue aldehydes in the rat kidney in varying degrees. Testosterone, on the other hand, brings about a deposition of tissue aldehydes in the ICM zone of the rat kidney or prevents the depletion caused by other steroids. Thus, for the first time, a specific influence of the sex hormones on one particular chemical tissue substance has been demonstrated in the rat, whereas, thus far, only such complex phenomena as organ growth and cellular response have been noted.

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[1] In view of the fact that aldehydes other than stearal and palmital may be present in tissues and because the name of plasmal given by Feulgen is too closely connected with these chemical units, the name of tissue aldehydes has been preferred in previous publications and in the present studies.

The modus operandi of these steroids on the metabolism of aldehydes is not clear. Nevertheless, a few definite conclusions can be drawn at this time. Oster (1945) demonstrated changes in the aldehyde content of the ICM zones of normal mature rats synchronized with the estrous cycle. This cyclic phenomenon effects not only a disappearance but also a reappearance of tissue aldehydes in the zone. When bilateral ovariectomy was performed on a mature rat, the ICM zone thereafter remained in that state of stainability which would correspond to the cyclic condition of the ovaries at the time of operation. Immature rats, on the other hand, have intensely stainable ICM zones which are maintained only if ovariectomy is performed before puberty. Administration of the principal female hormones to these animals, as suitable test animals, consistently resulted in a tissue aldehyde depletion, maintainable for reasonably long periods of time in contrast to the shorter duration of aldehyde depletion in the normal mature rat undergoing cyclic changes.

Therefore, the question arises as to what causes the reappearance of tissue aldehydes in the ICM zones of normal mature rats. A logical conclusion from the above findings would postulate the presence in the rat ovary of a hormone which causes this phenomenon of depositing aldehydes in the ICM zone. Whether this hormone be testosterone or a similar substance can be decided only by further studies.

What prevents the mouse kidney from exhibiting the same phenomenon as the rat kidney cannot be decided now. Our theory is that in some organ in the mouse is produced a substance which prevents the estrogens from exerting their influence on the tissue aldehydes in the ICM zone of the mouse kidney.

Ovariectomized animals, the kidney ICM zones of which have been depleted of aldehydes either by suitable steroids, diethylstilbestrol or during pregnancy, show an almost uniform tendency to revert very gradually to the original state of a deeply stainable zone. Since this restoring of aldehydes occurs in spite of the absence of the ovaries, one is forced to assume that some other organ may take over the function of the ovary to a minor degree in this respect and cause aldehydes to be again deposited in their original site.

A discussion of the physiological significance of the observed phenomenon can be only speculative at present. Disappearance of tissue aldehydes, which are components of the cell, by physiological and pharmacological influences would possibly cause a rearrangement in the equilibrium of the cell contents and in the maintenance of the cell membrane surface tension. One may then assume that such a disturbance, especially in the kidney, would alter the electrolyte balance, a condition often found in states of steroid hyper- or hypoactivity (premenstrual tension and edema of pregnancy). Thorn *et al.* (1938) describe a retention of sodium chloride and water in normal dogs with the injection of crystalline steroids. Electrolyte disturbance should

then be considered a secondary process in this case, following the primary effect of the steroids on the tissue aldehydes.

Selye and Rowley (1944) investigated the protection offered by large doses of methyl testosterone on experimental nephrosclerosis in rats caused by excessive administration of sodium chloride and desoxycorticosterone acetate. They suggest a renotropic effect of methyl testosterone which might be separable from the masculinizing properties of this hormone. It could very well be that this more unspecific protective action of testosterone is a specific one of tissue aldehyde deposition. These tissue aldehydes would in turn then prevent the occurrence of experimental nephrosclerosis.

Investigations of the influence of tissue aldehydes on the maintenance of normal blood pressure in the rat, as suggested by Oster (1942), the occurrence of the tissue aldehyde shift in other animal species and the influence of drugs on this phenomenon are in progress.

SUMMARY

The effect of the principal female and male sex hormones on the tissue aldehyde shift in the rat kidney was investigated with suitable test animals. It was found that estrogenic substances and progesterone exert a tissue aldehyde depleting influence and that testosterone exerts an aldehyde depositing or depletion preventing influence on the tissue aldehyde metabolism in the intercortico-medullary zone of the rat kidney.

Chemical studies indicate the presence of alcohol soluble and alcohol insoluble aldehydes in the hog kidney.

Mice do not exhibit a tissue aldehyde shift under conditions similar to the rat.

The presence of a substance in the rat ovary with testosterone-like action on the kidney tissue aldehydes has been postulated from the evidence of this investigation.

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SOME EFFECTS OF SEX STEROIDS ON THE GONADS OF THE STARLING[1,2]

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DESPITE many studies, the effects and actions of sex steroids on the avian gonad are not understood clearly. Moore and Morgan (1942) and Lahr and Riddle (1944) have summarized much of the literature. A bird such as the starling offers advantages for study because by photoperiodic manipulations the testis can be placed at a desired level of spermatogenetic activity whereby the annual cycle can be studied in its individual parts. A previous study on the starling (Burger 1944) found that testosterone delayed the response of the initially inactive testis to the stimulation evoked by "long days." This exogenous steroid did not effect spermatogenesis in testes which were already producing sperm. In the present study, some effects of estradiol and further effects of testosterone are reported. Of special interest is the response, novel for the bird, of the testis to estradiol.

MATERIALS AND METHODS

Table 1 records the various experimental treatments. Of the two light rations used, 14 hours daily light (long days) will induce complete spermatogenesis in the adult; 9 hours (short days) do not induce any spermatogenetic activity. The injections were made subcutaneously immediately anterior to the thigh. The gonads were preserved in Bouin's fluid and were stained with iron hematoxylin and eosin.

RESULTS AND DISCUSSION

A. Effects on the Ovary.

In the 21 days of experiment 1, no effects were produced on the sexually quiescent ovary by testosterone propionate and estradiol dipropionate. Neither steroid caused a change in the germinal or nongerminal elements. Ringoen (1940) found that theelin produced no effect in the inactive ovary of the English sparrow. The lack of response of the ovary of the starling apparently was not due to an inadequency of available steroid, since the oviducts markedly hypertrophied after treatment with estradiol.

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[1] The steroids used were supplied through the courtesy of Ciba Pharmaceutical Products, Summit, N. J.

[2] Aided by a grant from the Penrose Fund of the American Philosophical Society.

B. Effects of Estradiol on the Testis.

Testes of control birds responded in a fashion appropriate to the light treatments used. In experiment 2a, testes remained inactive; in experiment 2b, spermatids developed; in experiment 3, sperm formation continued. None of the testes from the birds treated with estradiol were like those of the controls. Three responses to estradiol were found: 1) There was a suppression of spermiogenesis and of the

TABLE 1. EXPERIMENTAL TREATMENTS

Expt. no.	Duration	No. of birds[1]	Dosage[2]	Daily light	Initial state of gonads
1a	11/19–1/9	6 ♀ ♀	1 mg. testosterone propionate[3]	9 hrs.	inactive
1b	11/19–1/9	6 ♀ ♀	1 mg. estradiol[3] dipropionate	9 hrs.	inactive
2a	11/19–1/9	3 ♂ ♂	1 mg. estradiol[3] dipropionate	9 hrs.	inactive
2b	11/19–1/9	5 ♂ ♂	1 mg. estradiol[3] dipropionate	14 hrs.	inactive
3	2/19–3/5	4 ♂ ♂	1 mg. estradiol	14 hrs.	sperm
4	3/6–3/19	4 ♂ ♂	1 mg. estradiol dipropionate, 1.5 mg. testosterone propionate	14 hrs.	sperm
5	1/29–2/12	4 ♂ ♂	1 mg. testosterone propionate	9 hrs.	spermatocyte

[1] An equal number of controls were used. All birds adult except in Expt. 1a, half were juveniles.
[2] 0.1 cc. sesame oil carried 1 mg. of steroid.
[3] Injected every 72 hrs., otherwise every 48 hrs.

multiplication of germ cells. Germ cells were destroyed, although all testes continued to retain primary spermatogonia. In some instances, even these cells were completely lacking in a local area of the testis. 2) There was a marked hypertrophy of intertubular tissue which in the normal starling is present only in small amounts. Where hypertrophy was most marked, the testes gave the appearance of being

EXPLANATION OF FIGURES

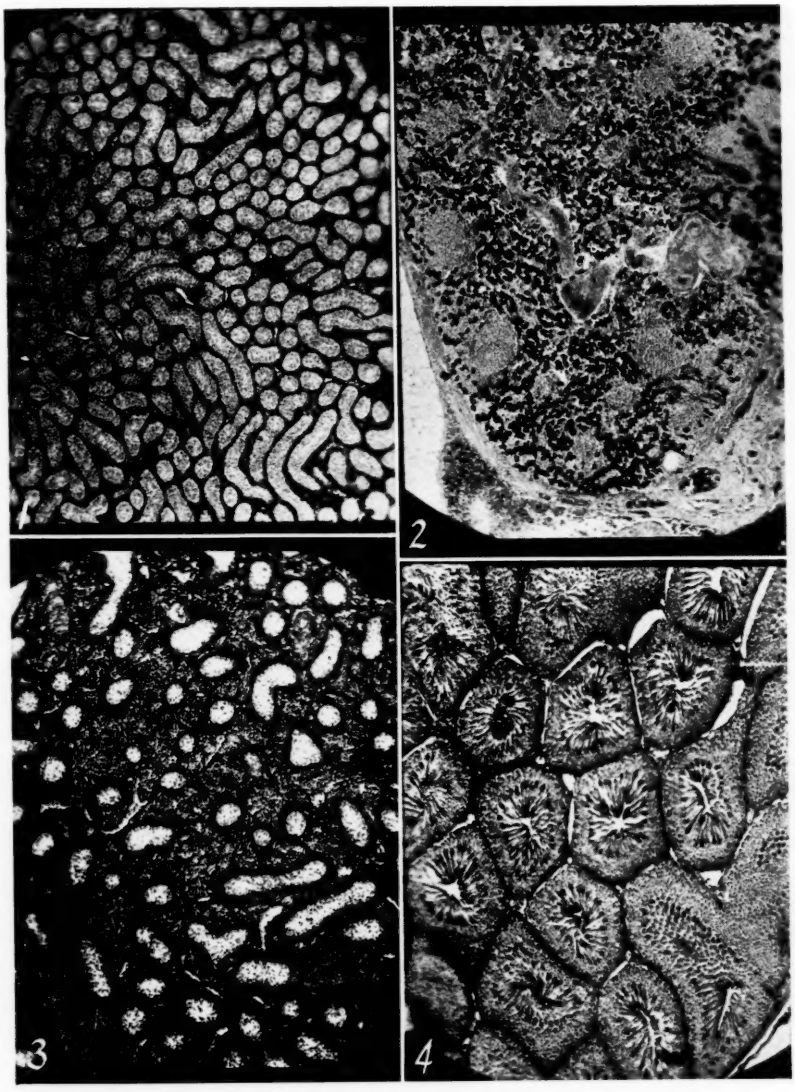
All figures are of sections of testes, ×90

FIG. 1. Normal sexually quiescent testis. Tubules form the bulk of testis. Conspicuous blood vessels not visible.

FIG. 2. 'Short day' bird after 21 days of estradiol treatment. Tubules are not visible in the figure, although remnants can be seen with the microscope. Local areas of this testis are sterile. Note the nodules of intertubular cells and the conspicuous arteriole running through the center of the figure. The black spots in the figure are pigment normally found in the starling testis.

FIG. 3. 'Long day' bird, initially sperm-forming after 14 days of estradiol. Scattered tubules widely separated by intertubular cells. Testis shown in figures 2 and 3 show the maximum modification effected in all the experiments. All other testes, however, showed the same type of modification.

FIG. 4. 'Long day' bird, initially with sperm, treated for 13 days with both estradiol dipropionate and testosterone propionate. This is in appearance a normal sperm-producing testis. Note the absence of intertubular tissue, and the thin walled blood vessels.



FIGS. 1–4.

made predominately of intertubular cells in contrast to the normal condition where the seminiferous tubules form the bulk of the gonad. In this hypertrophied tissue, eosinophilic (Leydig) cells became numerous. 3) The walls of the testicular arterioles also hypertrophied.

C. Histological Changes Following Estradiol Treatment.

While the normal testis undergoes tremendous volume changes during the annual sexual cycle, the intertubular cells are always inconspicuous (cf. figures 1 and 4). These cells consist of small patches and strands of connective tissue in which in the inactive testis a few eosinophilic cells can sometimes be found. In the sperm-producing testis eosinophilc cells are not seen. With massive doses of estradiol the intertubular tissue hypertrophies (figures 2, 3). This hypertrophy is due principally to fibrous cells which appear to separate from the walls of the tubules, and to gradually change from elongate compressed cells to irregular shortened cells with a clear cytoplasm. This type of cell next seems to become irregularly spherical or polyhedral and to develop eosinophilic cytoplasmic inclusions which come to fill the whole cytoplasm. These eosinophilic cells resemble Leydig cells.

Besides what appears to be a histological succession of stages from fibroblasts to eosinophilic cells, is the fact that in the sperm-producing testis, eosinophilic cells can not be found. Thus in experiment 3, there does not appear to be any Leydig cells or other cells free from the walls of the tubules or blood vessels from which the eosinophilic cells could come.

D. Combined Effects of Estradiol and Testosterone.

The simultaneous administration of testosterone and estradiol (experiment 4) neutralized within the duration of the experiment the adverse effects of estradiol alone. As seen in figure 4, spermatogenesis is normal and there is no hypertrophy of the intertubular cells. In one of the four treated birds, signs of slight damage to the sperm cells was found. It is of interest to mention that the testes of the treated birds were all larger than those of the controls. Two of the four birds had testes as large as any of the hundreds of starling testes we have examined.

E. Discussion of the Effects of Estradiol.

As far as the author is aware, this is the first report of hypertrophy of intertubular cells in the avian testis following treatment with estrogens. Lahr and Riddle (1944) found an atrophy of Leydig cells in the dove following treatment with estrone. Burrows (1935, 1936 reported hypertrophy in the interstitial cells of the mouse after estrogen, although Gardner (1937) did not get this effect with all strains of mice. In view of the numerous reports of various mesodermal deriva-

tives hypertrophying after estrogen treatment (cf. Allen, *et al.* 1939, Lipschütz, 1942 for summaries) the results in the starling are of interest. Since the rôle of the Leydig cell in the avian testis has not been established, the significance of the appearance of these cells after estradiol treatment is not known. Histological study confirms the conclusions of Pfeiffer and Kirschbaum (1943) that these cells come from fibroblasts.

The neutralization of the effects of estradiol by testosterone is like that secured by Pfeiffer and Gardner (1938) on the dove. In the starling a less high ratio of testosterone to estradiol is required to maintain spermatogenesis.

The adverse effects of estradiol seem due to a direct action by the steroid on the testis. In hypophysectomized animals generally, the testis does not become disorganized nor do the interstitial cells hypertrophy.

F. Effect of Testosterone on the Testis.

In testosterone treated starlings which were maintained on short days after their testes reached the spermatocyte stage, testes were larger than those of the controls. Control testes contained spermatids, while the testes from treated birds contained numerous sperm cells.

This and previous results throw some light on the role of male hormone in spermatogenesis. Since the work of Walsh, Cuyler, and McCullagh (1934), it is known that androgen prevents testicular atrophy and maintains spermatogenesis after hypophysectomy in some sexually active mammals, although substances other than androgen have this effect (Nelson and Gallagher, 1936; Hisaw, Greep, and Fevold, 1936).

In the English sparrow, Pfeiffer (in press) has found that males in active spermatogenesis and undergoing testosterone treatment suffer damage to spermatogenesis when the steroid is withdrawn. This damage can be repaired by renewed injections of androgen. Testosterone is not stimulating to the sexually quiescent testis. Pfeiffer has concluded that male hormone sustains and reinforces the later stages of spermatogenesis.

Our results lend support to this theory of Pfeiffer. In the absence of the continued hypophyseal stimulation produced by 'long days,' testosterone treated birds which were in the spermatocyte stage at the start developed further along in spermatogenesis than did controls. In the sperm-producing testis of 'long-day' birds, testosterone had no effect (Burger 1944), possibly because the testis itself was by now producing adequate hormone.

Testosterone does not augment spermatogonial multiplication, since it temporarily depresses the initially inactive testis even under 'long-day' treatment (Burger 1944). Its effect appears to be on the maturation rather than on the proliferation of germ cells.

SUMMARY

Estradiol dipropionate and testosterone propionate, with the dosage and duration of treatment used, had no effect on the sexually inactive ovary. Estradiol and its dipropionate modified testes which were either inactive or sperm-producing. The effects found were: 1) a suppression of spermatogenesis with a disappearance of all stages except the primary spermatogonial. Cells of this later stage became less numerous. 2) There was a marked hypertrophy of the intertubular tissue in which eosinophilic (Leydig) cells appeared. Cells for this hypertrophy appeared to come from the fibrous walls of the tubules. 3) There was a thickening of the walls of the testicular arterioles. Testosterone propionate neutralized the adverse effects of estradiol. The effects of estradiol seem to be due to a direct action on the testes.

Testosterone treated starlings which were initially in the spermatocyte stage and were maintained on 'short days' developed further into spermatogenesis than did controls. This result together with previous results lend support to the theory of Pfeiffer that in the bird, male hormone supports the maturation of male sex cells but not their spermatogonial multiplication.

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THE DISTRIBUTION OF CELL TYPES IN THE ANTERIOR HYPOPHYSIS DURING LATE PREGNANCY AND LACTATION

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WITHIN the last few years considerable evidence has accumulated from bio-assays (Hurst and Turner 1942) which shows that the lactogenic content of the anterior hypophysis increases markedly in mammals about the time of parturition. In some forms the increase has been observed during pregnancy, whereas in others no notable increase occurs until the time of parturition or soon afterwards. There have been relatively few cytological studies of the hypophysis from pregnant and lactating animals and attempts to correlate the cytology with assay results have been sadly neglected. Although Haterius (1932), and Desclin (1936) did not make differential cell counts, they reported that the hypophysis of the rat presents essentially the same picture during pregnancy and lactation. Weis (cited by Kirkman 1937) observed that in the guinea pig hypophysis the acidophiles increase in the first part of pregnancy, and that at parturition the acidophiles decrease while the basophiles increase in number. Collin and Florentin (1935) report that at parturition the numerous acidophiles in the guinea pig hypophysis during pregnancy transform into basophiles. Kirkman (1937), however, from his careful study of the guinea pig hypophysis, concluded that toward the end of pregnancy there is an increase in the number of acidophiles which attains a maximum soon after parturition, and that there was an accompanying drop in the percentage of basophiles. In view of the incomplete and conflicting work on this problem we have made differential cell counts of the anterior hypophysis from rats in various stages of pregnancy and lactation. It was hoped that an approach of this type would give an index of chromophile activity during pregnancy and lactation, and would perhaps indicate what cells are responsible for the elaboration of lactogen.

METHODS

All of the animals used in the experiment were healthy rats of the Long-Evans strain which were breeding for the first time. Since suckling is known to influence the lactogen content of the hypophysis the litters of the lactating

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groups were reduced to 5 on the day of birth. Rats were sacrificed on the tenth and twentieth day of pregnancy, and on the third, seventh, sixteenth and twenty-second day of lactation. Forty two animals, distributed according to Table 1, were used in the study. Differential cell counts were made on 39 specimens. All of the hypophyses were fixed in Bouin's fluid and stained with a modified Masson technique. Methods of sectioning and counting were the same as those previously employed by Baker and Everett (1944), except that in a few of the largest sections every fifteenth oil immersion field was counted instead of every tenth. The total number of cells counted in each hypophysis averaged 3513. The identity of the specimen was unknown to the investigator at the time he was making the count.

RESULTS

The relative percentage of acidophiles and the absolute number per field were essentially the same for both the ten and twenty-day

TABLE 1. HYPOPHYSEAL WEIGHT AND DISTRIBUTION OF CELL TYPES

Reproductive stage	No. Rats	Mean ratio Hyp. wt. / Body wt.	Mean percentage of cell types Acidophiles	Basophiles	Chromophobes	Mean no. per field Ac.	Ba.	Ch.
Pregnancy								
10 days	9	.047[1]	19.7±3.9[2]	6.4±1.5	73.8±4.1	25.5	8.4	96.6
20 days	7	.038[3]	19.4±4.3	4.4±1.4	75.9±3.9	26.3	6.2	103.1
Lactation								
3 days	7	.046[4]	36.9±6.4	3.2± .2	59.9±6.2	49.0	4.8	79.8
7 days	5	.043[5]	32.5±7.2	3.5± .8	63.8±7.3	44.6	4.9	87.5
16 days	5	.041	30.8±3.5	3.6±1.0	64.8±2.9	44.7	5.7	95.4
22 days	6	.039[6]	28.9±5.5	4.3±1.6	66.3±6.3	37.8	5.9	87.1

[1,3] Body wt. of pregnancy.
[2] Standard deviation.
[4,5] Six animals.
[6] Eight animals.

pregnant groups. By the third day of lactation the acidophiles had increased by almost 100%. Thereafter, during lactation, the percentage of acidophiles remained relatively high in comparison with the pregnant groups but there was a gradual decrease from the peak of 36.9% at three days post-partum (see table 1). Although the relative percentage of acidophiles at the sixteenth day of lactation was less than at the seventh day, the absolute number per field was practically the same.

Many of the acidophiles of the 10-day pregnant group were faintly granulated and in the 20-day group a greater majority of the cells were heavily granulated. There were no striking variations in the degree of granulation of these cells during the stages of lactation, although in general the granules were more numerous and more distinct than in the pregnant animals. In any one group, pregnant or lactating, there were acidophiles containing from few to many granules.

The basophiles were most numerous, both in relative percentage and number per field in the 10-day pregnant animals and lowest dur-

ing the third day of lactation. Although the differences are small, the basophiles became more numerous again as lactation continued.

Coincident with the great increase of acidophiles by the third day of lactation there was a reduction of the chromophobes, both in number per field and in the relative percentage. Furthermore, as lactation continued the absolute number of chromophobes tended to increase while the acidophiles decreased. However, at the sixteenth day of lactation the absolute number of chromophobes was considerably higher than during the twenty-second day of lactation. This discrepancy. was quantitatively comparable to the slight increase in the absolute number of acidophiles of this group above that of the 7-day group.

DISCUSSION

In view of the sudden increase in lactogenic content of the hypophysis after parturition, one would expect either a sudden enlargement of the gland, an increase in granulation of cells producing lactogen, or an increase in their number as a result of mitotic division or conversion from chromophobes. The mean ratio of the hypophysis weight divided by the body weight indicates that there is no marked enlargement of the rat hypophysis at the termination of pregnancy or during the first few days of lactation. Determination of this ratio for the pregnant rats by using the body weight at the time of insemination does not alter the pattern given in the table. Likewise, comparison of the mean absolute weights gives essentially the same picture. This relatively constant weight of the hypophysis agrees with the findings of Holst and Turner (1939) for the guinea pig. However, the same workers noted a marked increase in weight of the hypophyses from 10-day post-partum rabbits over that of non-lactating animals. Hurst and Turner (1942) found that the hypophyses from lactating mice generally weighed less than the glands from pregnant mice. Furthermore, Reece and Turner (1936) record smaller weights for hypophyses of 21-day pregnant rats than for 12-day pregnant animals. Thus, in general, the hypophysis seems to vary little in weight during pregnancy and lactation.

Wolfe and Cleveland (1933) reported that there is a reduction in granulation of acidophiles in the rat hypophysis during the first third of pregnancy and an increase in granular material during the last third. This agrees with our finding of an increase in acidophilic granulation during the latter part of pregnancy and the first part of lactation. This observation and the appearance of many well granulated cells during lactation suggests that these granules are related to the storage of lactogen. Since all degrees of granulation were observed in any one stage we have no evidence to show whether it is the heavily granulated or faintly granulated cell which is liberating the hormone.

The most significant change noted in the anterior hypophysis of

the rat in the course of this study was the great increase in the acidophiles three days after parturition. This increase, almost 100% over the number present in the pregnant groups, is without doubt related to the elaboration of lactogen. Assays of hypophyses from the rat, rabbit, mouse, guinea pig, and goat at different periods in the reproductive cycle reveal that the lactogen content is highest soon after parturition. (For summary see Hurst and Turner, 1942). Furthermore, the lactogen content of the rat, guinea pig, and rabbit hypophysis is relatively constant during pregnancy and in all there is a sharp rise after parturition. As lactation progresses the lactogen content of the hypophysis gradually declines. A curve showing the percentage of acidophiles present in the hypophysis during pregnancy and lactation, as we have recorded, would closely parallel the curve for lactogen content of the rat at these same stages.

Kirkman (1937) found that in the guinea pig the acidophiles increased in number toward the end of pregnancy and attained a maximum soon after parturition. This increase was accompanied by a drop in the percentage of basophiles. He also observed an increase in the number of basophiles near the middle of pregnancy and possibly a second increase near the end. We have no figures for the percentages of cell types at the beginning of pregnancy but we found the percentage of basophiles to be highest in 10-day pregnant rats which agrees with Kirkman's observation. Therefore, increased granulation of acidophiles and increase in their number are the significant changes to be correlated with the increase in lactogen content.

Our figures for the percentages of acidophiles present during pregnancy are somewhat lower than those of Wolfe and Cleveland (1933) for the rat who report 31.7% for animals of the second third and 32.8% for those in the last third of pregnancy. Contrary to their observation that basophiles increase in the latter part of pregnancy, we observed a slight decrease.

Stein (1934) from quite an extensive study of the rat hypophysis during all stages of pregnancy concluded that there was no significant difference in the percentage of cell types present in pregnant and nonpregnant animals. His percentages for acidophiles was higher than ours, but lower than those of Wolfe and Cleveland. In contrast to these investigators we used a technique which destroys mitochondria and sharply differentiates acidophiles from chromophobes. This could very well account for our lower acidophile count. Also, these differences in percentages of acidophiles from the three studies, all of which show the same trend, are perhaps due in part to differences in interpretation by the respective observers. Thus, it is apparent that little emphasis should be placed upon absolute values in making comparisons.

Other investigators have contributed evidence in support of the individuality of hypophyseal cell types in the elaboration of hormones.

Friedman and Hall (1941) and Azimov and Altman (1938) found lactogen to be more concentrated in the acidophilic zone of the beef hypophysis. Similarly, Schooley and Riddle (1938) associate the acidophiles of the pigeon hypophysis with the elaboration of lactogen. They made no differential cell counts, but they report that the acidophiles increase in number during the first week of incubation to the point where sparsely granulated ones predominate and that this condition is maintained until the end of feeding the young. They also found that the basophiles undergo further atresia during incubation and the feeding period.

Thus, our finding of an increase in number and granulation of acidophiles coincident with a reduction in number of basophiles during the period of great increase in lactogen indicates that the acidophiles and not the basophiles are probably responsible for the production of lactogen.

No statistical treatment was made of the mitotic figures observed in this study, however, relatively few dividing cells were seen in any of the preparations. There was no apparent increase in the mitotic rate in any cell type from any of the six groups. Thus, it follows that the great increase in acidophiles must be at the expense of other cell types. The decrease in the mean number of chromophobes per field as the number of acidophiles increases suggests that the acidophiles develop from chromophobes. The very slight decrease in the mean number of basophiles per field could not possibly account for the acidophilic increase. Furthermore, there is no evidence from our work that basophiles transform into acidophiles.

The total mean number of cells per field remains rather constant except for the 16-day lactating group which shows about 9 cells more per field than any of the others. We are unable to offer an explanation for this increase.

SUMMARY

Differential cell counts of the hypophysis from pregnant and lactating rats show that at 3-days post-partum the acidophiles have increased by almost 100% above the number present in pregnant animals. This number decreases as lactation continues but does not revert to the pregnancy level. The increased number of acidophiles and their increased granulation is believed to be associated with the high lactogen content of the hypophysis. This increase of acidophiles was due to conversion of chromophobes and not from division of acidophiles.

Basophiles are more numerous in 10-day pregnant rats than at 20-days pregnant or during lactation.

There is no significant weight change in the rat hypophysis during lactation as compared with pregnancy.

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PROSTATE AND SEMINAL VESICLE RESPONSE TO TESTOSTERONE PROPIONATE IN INTACT AND CASTRATE RATS[1]

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IN A PREVIOUS COMMUNICATION (Krichesky, Benjamin, Belt and Schwartz, 1941) it was reported that testosterone propionate injections into intact, completely prostatectomized rabbits produced a rapid increase in the surface area of intraocular prostatic implants which reached a peak in from 10 to 16 days and was then followed by regression to the pre-injection level in six to eight weeks. On the other hand, such implants in completely prostatectomized, castrate rabbits responded to testosterone propionate injections by a gradual and continuous increase in surface area for as long as 95 days (the duration of the experiment). The immediate objective of the present experiments was to determine if similar responses of the prostate gland could be demonstrated in intact and castrate rats.

MATERIALS AND METHODS

A total of 128 male rats were used; all were of the Wistar strain and ranged from 125 to 155 days of age. They were fed the Stienboch diet with fresh cabbage added twice a week. Control and experimental animals were kept in separate cages. The animals were grouped as follows: 41 castrate experimentals, 41 intact experimentals, 31 intact controls and 10 castrate controls. Litter-mates were opposed as much as possible among the four groups.

Testosterone propionate in sesame oil was injected intramuscularly three times weekly.[2] The dosage was one mg. per kg. body weight. The first injection into castrate animals was given less than 24 hours after gonadectomy.

Animals from each group were sacrificed at intervals of one, two, four and seven weeks. The entire genital system was removed, fixed in Bouin's fluid and stored in 70 per cent alcohol. Subsequently, the prostate gland and seminal vesicles were cleaned of connective tissue and weighed on a torsion balance. After total prostate weight was obtained, the lobes of the prostate were separated from one another and the weight of each lobe was determined.

All data are expressed as per cent change in organ-body weight ratios.

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[2] The testosterone propionate used was supplied to us through the kindness of Dr. Ernst Oppenheimer, Ciba Pharmaceutical Products Inc., Summit, New Jersey.

Changes in organ body-weight ratios were obtained for the total prostate, for each lobe of the prostate, for the seminal vesicles and for the testes.

RESULTS

The data summarizing the results of these experiments are given in table 1. These indicate that at the end of each injection period the prostates of both intact and castrate animals were enlarged when compared with the prostates from uninjected castrate and intact controls (Fig. 1). Moreover, the prostates from intact animals were

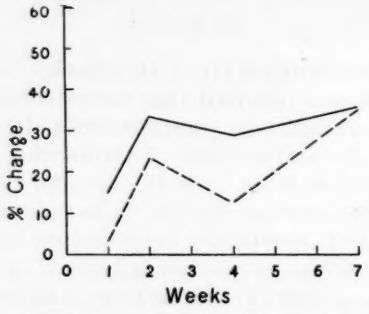

FIG. 1. Per cent change in total prostate-body weight ratio. Intact animals represented by solid line, castrate animals by broken line.

larger at all times than those of the castrates at comparable periods. In intact injected animals the hypertrophy appeared to almost reach a maximum (33.7 per cent over the intact controls) by the second week, falling to 28.7 per cent by the fourth week and rising again to 35 per cent by the seventh week. In castrate animals, hypertrophy of the prostate gland remained below but parallel to that in the intact animals through the fourth week. By the seventh week the per cent

TABLE 1. PER CENT CHANGE IN ORGAN-BODY WEIGHT RATIOS OF PROSTATE AND SEMINAL VESICLE IN INTACT AND CASTRATE RATS INJECTED WITH TESTOSTERONE PROPIONATE

No. of Animals	Average Weight	Weeks	Total Prostate	Ventral Lobe	Middle Lobe	Anterior Lobe	Seminal Vesicle	Testes
			Intact Animals[1]					
8	242	1	14.2	16.0	13.5	4.2	24.5	3.3
8	259	2	33.3	28.0	22.0	22.0	64.5	−11.5
12	225	4	28.7	18.0	48.5	31.7	52.0	−21.3
13	293	7	35.4	54.7	54.2	22.0	27.7	−48.0
			Castrate Animals[1]					
8	230	1	3.3	−0.67	16.5	2.1	16.2	—
8	258	2	23.5	21.0	10.5	29.3	27.7	—
13	253	4	12.4	4.5	23.6	23.6	32.1	—
12	262	7	35.0	25.9	51.0	52.8	47.5	—

[1] Average organ-body weight ratios for the controls was considered as 0.0 per cent.

increase in organ-body weight ratio approached that of the intact animals (35 per cent over the castrate controls).

The response of the various lobes of the rat prostate to injected androgen was considered separately and the results are included in table 1. The terminology used was adopted from Moore, Price and Gallagher, 1930.

The response of the ventral lobe of the prostate (Fig. 2) was similar in some ways, and differed in others from the response of the total prostate. The similarities were: (a) the per cent increase in the intact group was consistently above the increase in the castrate group; (b) a regression in ventral prostate weight occurred in both intact and castrate animals between the second and fourth weeks; and (c) a maximum increase was reached in both groups over their respective controls at seven weeks. The differences in the response of

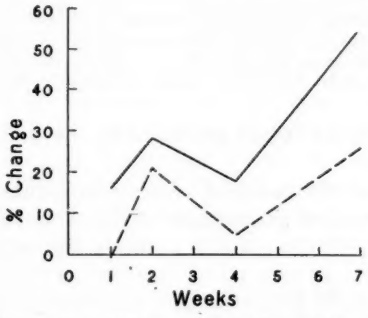

FIG. 2. Per cent change in ventral prostate-body weight ratio. Intact animals represented by solid line, castrate animals by broken line.

the ventral prostate from the response of the total prostate are: (a) organ-body weight ratios for the ventral lobe in the intact animals showed no tendency to plateau as was indicated by the response of the total prostate; and (b) at seven weeks the per cent increase in organ-body weight ratio for the ventral lobe was much greater in the intact group than in the castrate group (54.7 per cent in intact animals as compared with 25.9 per cent in castrate animals).

The middle lobe of the prostate responded by a continuous increase in organ-body weight ratio in the intact animals. Except for a slight regression between the first and second week the response in the castrate group was similar. For the most part, the hypertrophy was less in the latter group than in the former (Fig. 3) but by the seventh week the per cent increase was slightly over 50 per cent for both.

The response of the anterior lobe to injected testosterone propionate differed markedly from the response of any of the other lobes (Fig. 4). In both the intact and castrate groups the per cent change was similar at the end of the first second and fourth week. After the

fourth week the anterior lobe in the intact group underwent regression while the same lobe in the castrate group showed marked enlargement. At seven weeks the organ-body weight ratio was only 22.0 per cent

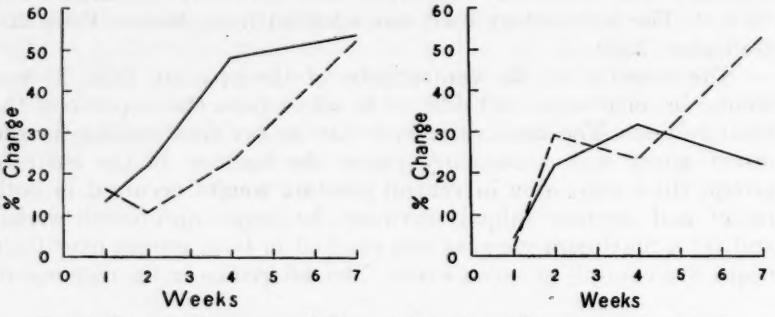

FIG. 3. Per cent change in middle prostate-body weight ratio. Intact animals represented by solid line, castrate animals by broken line.
FIG. 4. Per cent change in anterior prostate-body weight ratio. Intact animals represented by solid line, castrate animals by broken line.

over the controls in the intact group while it reached 52.8 per cent in the castrate group.

The response of the seminal vesicles in intact and castrate rats to injected testosterone propionate differed markedly from that of the prostate (Fig. 5). In intact animals the seminal vesicle-body

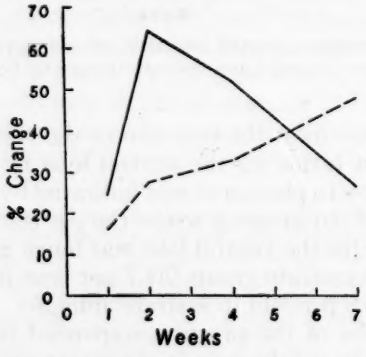

FIG. 5. Per cent change in seminal vesicle-body weight ratio. Intact animals represented by solid line, castrate animals by broken line.

weight ratio increased precipitously, reaching a peak of 64.5 per cent over the intact controls by the end of two weeks. Thereafter, continuous regression occurred until the seventh week when the organ-body weight ratio was only 27.7 per cent above the controls. In castrate animals, however, the organ-body weight ratio increased uninter-

ruptedly throughout the course of the experiment to reach a maximum of 47.5 per cent over the castrate controls by the seventh week. The seminal vesicles of the intact animals were larger than those of the castrates at the end of one, two and four weeks of injection, but this relationship was reversed by the end of the seventh week.

With the dosage of testosterone propionate employed, there was regression of the testes in the intact animals (cf. Table I). By the end of the second week a decrease of 11.5 per cent was noted and this fall in organ-body weight ratio continued to 48.0 per cent by the end of the experiment. The epididymis was dissected only in animals sacrificed at four and seven weeks. The weight of the epididymis was 5.8 per cent less than in uninjected controls after four weeks and 30.0 per cent less after seven weeks.

DISCUSSION

The dosage of testosterone propionate used in these experiments (one mg. per kg. body weight, three times weekly) maintained the prostate and seminal vesicle weights above uninjected control animals in both intact and castrate male rats. These results are in agreement with those of Zuckerman (1936), Korenchevsky and Hall (1939), Korenchevsky, Hall and Ross (1939) and others. Consideration of the three lobes of the prostate of the rat separately indicates differences in response of these lobes to injected androgens. The responses of the ventral and middle lobes are, in general, somewhat similar (cf. Figs. 2 and 3), and, except for the absence of regression of the middle lobe, which occurred at the end of the fourth week, the curves representing changes in organ-body weight ratios for these two lobes parallel closely the curves showing total prostate response. The anterior lobe, on the other hand, shows a dissimilar pattern of response in that during the first four weeks anterior prostate enlargement in both castrate and intact animals is approximately equal and, in general, in the same direction. By the end of the seventh week the anterior lobe has regressed in the intact animals but has undergone continued hypertrophy in the castrate animals. It must be admitted that the small number of animals in each group, the difficulty in separation of each lobe from the others, and the small size of each lobe makes these values of doubtful significance so that a more accurate index of prostate response is obtained by consideration of total prostate response.

It is interesting to note that the response of the seminal vesicle differs markedly from the response of the total prostate. Whereas the prostate reaches its greatest hypertrophy at seven weeks in both intact and castrate animals, the seminal vesicle in intact rats undergoes marked regression after a peak of enlargement at two weeks, while in castrate animals this organ undergoes a continuous

hypertrophy throughout the course of the experiment. The response of the seminal vesicle in rats, but not the prostate, is similar to that reported for the prostate in rabbits (Krichesky, *et al.*, 1941).

No adequate explanation can be offered for the difference in the response of the prostate and seminal vesicle in the rat to injected androgens. It has been suggested by Korenchevsky and his co-workers, and others, that testosterone propionate inhibits gonadotropin secretion of the pituitary with a consequent suppression of normal testicular secretion, but that this androgen is sufficiently potent to produce stimulation of the secondary sex organs, even greater than normal. This hypothesis may explain satisfactorily the changes obtained in the rat prostate but does not explain the regression of the rat seminal vesicle in intact animals while the castrates exhibit continuous hypertrophy of the seminal vesicle. The total circulating hormone in the intact animals should be the same or even greater than in castrate animals. In the former there is present only the endogenous androgens, presumably of adrenal cortical origin, and the injected exogenous androgen, while in the latter (intact animal) the total circulating hormone would be the same and, in addition, any androgenic hormone of testicular origin. It would be expected then that the response of the seminal vesicles in intact animals would be greater to injected androgens than in castrates receiving the same dose. The data reported here do not support this hypothesis.

Two suggestions may be made to explain the results obtained. The difference in response of the prostate and the seminal vesicles may be due to differences in threshold of the two organs. It is known that weaker androgens or even strong androgens in very small doses may result in regression rather than stimulation of the secondary sex organs. Regression of the prostate after administration of weak androgens has been reported by Korenchevsky, Dennison and Kohn-Spayer (1833), Moore and Price (1937) and Korenchevsky and Hall (1939). Regression of comb weight in intact chicks has been reported after very small doses of testosterone propionate by Breneman (1942). A lower threshold of the prostate may account for the maintenance of the prostate at a high level of hypertrophy while a high threshold of the seminal vesicle may account for the failure to maintain hypertrophy in the latter organ.

An alternative suggestion is one which assumes a response of the seminal vesicle to decreased hormone concentration similar to that observed in the endometrium. In the castrate animal the amount of circulating hormone remains nearly constant throughout the course of the experiment and the target organ is subjected to a nearly constant stimulation. But in the intact animal the concentration of circulating hormone is greatest during the early period of the experiment and falls once the production of gonadotropin is sufficiently reduced to suppress testicular secretion. The reduction in the concentration of

circulating androgen may result in a regression of the seminal vesicle similar to the regression seen in endometrium on reduction of the concentration of circulating oestrogens (Markee, 1940). The problem of target organ response in relation to differences in threshold and to changes in concentration of circulating hormone requires further elucidation.

The injurious effect of injected testosterone propionate on the testes observed in this experiment is in agreement with the findings of Price (1936), Moore and Price (1938) and others. As suggested by some of these investigators this deleterious effect on the testes is probably an indirect one mediated through inhibition of the hypophysis.

SUMMARY

Castrate and intact male rats have been treated with testosterone propionate for periods varying from one to seven weeks. The per cent changes in organ-body weight ratios were determined for the total prostate, the anterior, middle and ventral lobes, the seminal vesicle and the testes. Organ-body weight ratios were maintained at or above control levels in both castrate and intact animals in the case of both the prostate gland and seminal vesicle. Testes weight decreased markedly throughout the course of the experiments.

In general, the response of the prostate gland and its various lobes was similar in both intact and castrate animals. In the case of the seminal vesicle, however, there was a marked difference in response in the castrate from that in the intact animals. In the former, the seminal vesicle responded by a continuous and uninterrupted hypertrophy during the course of the experiment while in the intact animals the seminal vesicle reached a maximum hypertrophy at two weeks, followed by continuous regression until the end of the experiment.

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THE CHOLESTEROL AND ASCORBIC ACID CONTENT OF THE ADRENAL, LIVER, BRAIN, AND PLASMA FOLLOWING HEMORRHAGE

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INTRODUCTION

THE FACT that many features of the shock syndrome are not unlike those of adrenal cortical insufficiency has suggested to some investigators (Swingle *et al.*, 1933) the possibility that the development of shock following injury may be the result of the failure of the secretory activity of this organ. Certainly the gross hemorrhagic appearance, together with the depletion of the stainable lipid of the gland, indicate that the adrenal undergoes intense stimulation during shock. Furthermore, loss of the adrenals renders the animal hypersensitive to injury, a defect which may be, in part at least, corrected by cortical hormone therapy. That lack of cortical hormone is, however, a limiting factor in the survival of the intact animal in shock has not been definitely established. Swingle and Remington (1944) have reviewed the great deal of conflicting data on this subject and have come to the conclusion that there has been no clear demonstration that cortical extract can protect against the development of shock in the intact animal. In this uncertain state of our knowledge regarding the rôle of the adrenal cortex in shock, it is of some interest to obtain quantitative data on metabolic processes known to be associated with the functional activity of this gland. The changes in two adrenal constituents, cholesterol and ascorbic acid, following hemorrhage have been studied. Evidence has previously been presented (Sayers *et al.*, 1944a, Sayers *et al.*, 1944b) that both of these substances are either directly or indirectly concerned with the elaboration of the secretions of the adrenal cortex. The adrenotrophic hormone of the anterior pituitary, a hormone in whose absence the adrenal cortex atrophies and the response of the organism to stress is markedly disturbed, brings about dynamic changes in both the cholesterol and ascorbic acid content of the adrenal (Sayers *et al.*, 1944a, Sayers *et al.*, 1944b). During the period in which the cholesterol content of the

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organ is depleted, gluconeogenesis goes on at an accelerated rate in the liver, a phenomenon which has also been shown to occur following administration of cortical steroids (Long, Katzin and Fry, 1940). This fact, together with the close structural relationship between cholesterol and the cortical hormones, suggests that cholesterol is the precursor of the cortical hormones and is depleted from the gland whenever the secretory activity of the organ is accelerated, as, for instance, after administration of adrenotrophic hormone or following the application of a wide variety of external or internal stimuli (Sayers *et al.*, 1944a).

Thus, earlier work has shown that in shock or following exposure to stress, histological and chemical changes take place in the adrenal. Areas of hemorrhage, congestion and dilatation of small vessels, along with an infiltration of polymorphonuclear leucocytes, have been described (Dunphy, Gibson and Keeley, 1941; Duncan and Blalock, 1942). The stainable lipid of the cortex is depleted after severe hemorrhage in man (Elliot, 1914) and in the rat (Engel, Winton and Long, 1943), in traumatic shock in the dog (Donahue and Parkins, 1935), in freezing shock in the dog (Muirhead *et al.*, 1942), and following burns in the human, the rabbit (Urechia, Manta and Bumbacescu, 1940) and the guinea pig (Pfeiffer, 1920).

A depletion of adrenal cholesterol after burns in the guinea pig has been reported by Crema (1928) while Harkins (Harkins and Long, in press), working in this laboratory, has observed a marked depletion of adrenal cholesterol in the rat after scalds inflicted by hot water. Clark and Rossiter (1944) have found that the ascorbic acid content of the adrenal, but not that of the liver, is depleted in the rabbit following a severe, but not fatal, burn. While this work was in progress Popjak has reported (1944) that following shock produced in rats by limb tourniquets, there was an extensive depletion of adrenal cholesterol largely confined to the ester fraction. In animals surviving twenty-four hours, the glands underwent hypertrophy. The other lipid fractions of the adrenal were unchanged, but the staining reaction with phenylhydrazine was increased, indicating an increased content of cortical steroid hormones. The author concludes that cholesterol is the precursor of these hormones.

These studies have included hypophysectomized animals, for if the thesis is correct that in the intact animal it is the pituitary which regulates the secretory activity of the adrenal cortex, then it is to be expected that the cholesterol and ascorbic acid of the adrenal would remain inert following subjection of the hypophysectomized animal to bleeding. The data have a bearing not only on the response of the animal to hemorrhage but also on the resistance of the organism to stress in general.

It was of some interest to know whether the changes in cholesterol and ascorbic acid were specific for the adrenal cortex. These two con-

stituents, therefore, have been determined in the liver, brain and plasma, as well as in the adrenal.

The report consists of two parts. Part one is concerned with the response of the rat to a non-fatal hemorrhage, a stress from which the animal can successfully recover. Part two deals with the metabolic changes in the organism subjected to a hemorrhage leading to shock and death. Great care has been taken to standardize both types of bleeding, for many discrepancies in the literature, with regard to metabolic changes following hemorrhage, may very well be due to lack of uniformity in the degree of severity of the hemorrhage.

METHODS

The animals used were male rats from the Sprague-Dawley colony. They were kept at an environmental temperature of $73 \pm 3°$ F. for at least 7 days previous to fasting. During the 24-hour fasting period and throughout the subsequent experimental period an environmental temperature of $84 \pm 1°$ F. was maintained. No anesthesia was used at any time during the experimental period. All control animals were also fasted for 24 hours at an environmental temperature of $84 \pm 1°$ F.

Animals weighing 85 to 150 gm. were subjected to a severe but non-fatal hemorrhage which consisted in the removal of an amount of blood by tail vein equal to 1.1 ml. per 100 square centimeters of body surface area in one hour. When hypophysectomized rats were used, the pituitary was removed three days previous to the bleeding. The animals were sacrificed at 0, 3, 6 and 24 hours following the end of bleeding.

For the study of the changes associated with a hemorrhage leading to shock and ultimate death, male rats weighing 240–260 gm. were bled from the tail veins to an extent of about 3 per cent of their body weight in an hour. After such a severe hemorrhage most animals will collapse and die within an hour after the cessation of bleeding. However, in order to prolong the period in which the animals were in shock, they were transfused through the tail vein at the time of this initial respiratory collapse with an inadequate volume of blood. The quantity of this transfusion was so adjusted that a second collapse occurred in less than three hours. At this time a second transfusion is given sufficient to completely replace the blood originally withdrawn. In spite of the total replacement of the blood volume, all the animals so treated remained in shock and died within a few hours. Fuller details of the standardization of the technique will be published shortly.

The rats in severe shock were autopsied in two groups. The first was sacrificed at an intermediate stage which was arbitrarily set at one hour following the second transfusion of blood (intermediate stage) while the second was sacrificed at the time of the final respiratory collapse (terminal).

The ascorbic acid content of the tissues was determined by the method of Bessey (1938) and total cholesterol by the method of Schoenheimer and Sperry, as described by Sperry (1938). At the end of the experimental period the animals were anesthetized with nembutal and the tissues promptly removed for analysis. One adrenal was analyzed for total cholesterol, the other for ascorbic acid. The adrenals were crushed and extracted in 15 ml. centrifuge tubes. The liver and brain were divided into approximately two equal

parts. One half was ground and extracted in a mortar with an alcohol-acetone mixture, the other half ground in a mortar with three per cent metaphosphoric acid. The adrenals were weighed on a torsion balance to the nearest 0.1 mg., the liver and brain on an analytical balance to the nearest mg. Blood was withdrawn from the inferior vena cava and run into tubes containing four mg. of potassium oxalate and four mg. of sodium fluoride for each ml. of blood. The blood was centrifuged for 10 minutes at 2000 R.P.M. Within 20 minutes after withdrawal of the blood from the animal the plasma was mixed with the alcohol-acetone mixture for the determination of total cholesterol[2] and with metaphosphoric acid for the determination of ascorbic acid. Plasma amino nitrogen was determined by the method of Frame, Russell and Wilhelmi (1943), plasma sugar by a modification of Folin's micro method adapted to the photoelectric colorimeter (Rubicon Company, 1943).

RESULTS

Effects of Non-fatal Hemorrhage

A volume of blood equal to 1.1 ml. per 100 square centimeters of body surface (about one half the volume of a fatal hemorrhage) was

TABLE 1. ADRENAL, LIVER, BRAIN AND PLASMA CHOLESTEROL FOLLOWING A NON-FATAL HEMORRHAGE

Values expressed as mg. per 100 mg. of fresh tissue and mg. per 100 ml. of plasma. Each value represents average and standard error of 4 to 6 animals.

	Controls (not bled)	Period following end of bleeding in hours 0	3	6	24
Adrenal					
Intact	6.18±0.335	4.23±0.615	2.97±0.458	2.76±0.393	4.66±0.374
Hypophysectomized	6.55±0.434	6.11±0.645	6.71±0.808	7.38±0.711	—
Liver					
Intact	0.338±0.014	0.313±0.038	0.318±0.012	0.333±0.014	—
Hypophysectomized	0.418±0.053	0.399±0.033	0.480±0.047	0.423±0.047	
Brain					
Intact	1.86±0.025	1.81±0.012	1.77±0.034	1.82±0.016	—
Hypophysectomized	1.81±0.058	1.77±0.057	1.80±0.038	1.81±0.016	—
Plasma					
Intact	60±1.5	53±3.5	49±3.5	54±4.3	65±1.1
Hypophysectomized	72±4.2	64±4.2	66±5.5	71±8.9	—

removed in one hour. Even hypophysectomized animals were in fairly good condition when subjected to this degree of hemorrhage. An elevation of plasma amino nitrogen which has been shown to be a characteristic feature of hemorrhagic shock in the rat (Engel, Winton and Long, 1943) was not observed in either the intact or hypophysectomized animals. The technic has been sufficiently characterized to make it quite clear that these animals were not in a state of shock.

Adrenal. A blood loss equivalent to 1.1 ml. per 100 square cms. of body surface produces a striking decrease in the cholesterol content of the adrenal of the intact rat. This is shown in Table 1. During the period of one hour in which the blood was removed, the concentration

[2] The values obtained for total cholesterol in the plasma of oxalated blood are 15 per cent too low, due to the effect of oxalate in altering the cell and plasma volume (Sperry and Schoenheimer, 1935).

of cholesterol decreased by 32 per cent, three hours after bleeding by 52 per cent, and six hours after bleeding by 55 per cent. In animals sacrificed 24 hours after bleeding, during which time they had access to water but no food, the concentration of cholesterol increased and approached the initial control level. These changes, together with those following the administration of adrenotrophic hormone of the anterior pituitary to normal rats, are shown in Figure 1. The striking parallelism between the two curves in this figure is at once evident

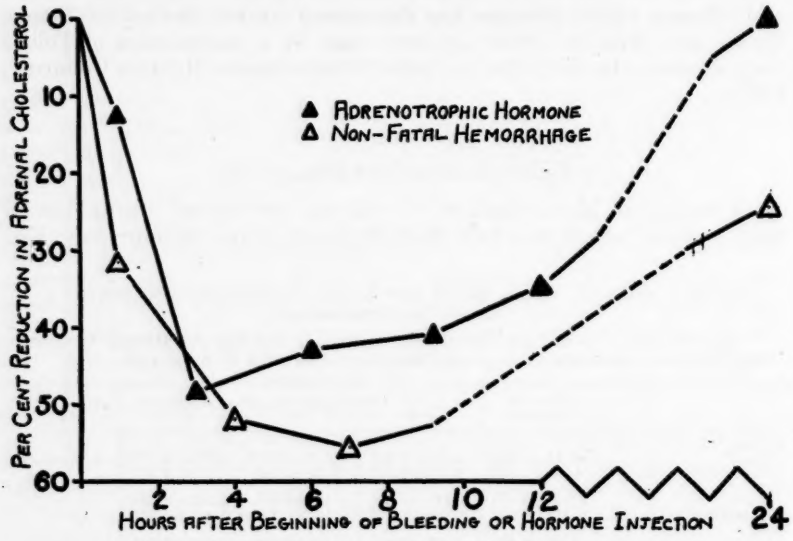

Fig. 1. Depletion of adrenal cholesterol following a non-fatal hemorrhage and following the administration of a single dose of adrenotrophic hormone.

and supports the hypothesis that the change in cholesterol in the adrenal of the intact animal following bleeding is brought about by the increased secretion of the adrenotrophic hormone of the pituitary. Furthermore, when the pituitary is removed, the cholesterol of the adrenal becomes inert and no longer undergoes a decrease when the animal is subjected to bleeding. This is illustrated in Table I, where it can be seen that no significant difference exists between the concentration of cholesterol in the adrenal of the control hypophysectomized animals and hypophysectomized animals sacrificed zero, three or six hours following bleeding.

The ascorbic acid content of the adrenal of the intact rat is also decreased following bleeding. However, the nature of this change is such that both phases, that of depletion and that of recovery, take place at a more rapid rate than they do in the case of cholesterol. At

the end of the bleeding period the concentration of ascorbic acid in the adrenal of the intact rat has been decreased by 58 per cent (Table 2), three hours later by 37 per cent, and six hours later by only 27 per cent. In animals allowed to recover 24 hours the concentration of ascorbic acid in the adrenal is actually higher than the initial control level. These changes, together with those following the administration of adrenotrophic hormone, are illustrated in Figure 2. Again, as in the case of cholesterol, the striking similarity as to the absolute amount of the changes and the time relationships strongly suggest that in the intact animal it is the anterior pituitary which initiates

TABLE 2. ADRENAL, LIVER, BRAIN AND PLASMA ASCORBIC ACID FOLLOWING A NON-FATAL HEMORRHAGE

Values expressed as mg. per 100 gm. of fresh tissue and mg. per 100 ml. of plasma. Each value represents the average and standard error of 4 to 6 animals.

	Controls (not bled)	Period following end of bleeding in hours 0	3	6	24
Adrenal					
Intact	398±17.0	166±6.4	251±31.5	292±20.0	456±7.2
Hypophysectomized	356±20.2	398±24.1	385±23.2	378±25.5	—
Liver					
Intact	18.2±0.91	22.6±0.44	23.2±0.61	24.3±0.99	26.5±1.50
Hypophysectomized	17.8±2.21	22.5±1.29	26.6±1.01	26.0±1.62	—
Brain					
Intact	40.6±0.96	39.8±0.63	40.0±0.50	40.5±0.24	—
Hypophysectomized	40.0±0.76	38.3±0.59	40.5±1.06	38.9±0.76	—
Plasma					
Intact	0.81±0.105	1.43±0.072	1.27±0.114	0.93±0.051	0.79±0.077
Hypophysectomized	0.70±0.163	0.94±0.110	0.81±0.073	1.10±0.128	—

these processes. This concept is supported by the observation that removal of the pituitary leaves the ascorbic acid as well as the cholesterol content of the adrenal inert and non-responsive when the animal is subjected to stress. In Table 2 it can be seen that no significant difference exists between the concentration of ascorbic acid in the adrenals of control hypophysectomized rats and those hypophysectomized animals sacrificed zero, three and six hours after the end of bleeding.

The intact animals subjected to the non-fatal hemorrhage responded with a hyperglycemia. This is part of a well known sympathetic reaction of the organism to stress. The association of the dynamic changes in adrenal cholesterol and ascorbic acid just described with this hyperglycemia in the intact rat is illustrated in Figure 3. The hypophysectomized animal, on the other hand, failed to mobilize glucose for any prolonged period. With this failure is associated the inert nature of adrenal cholesterol and ascorbic acid in this animal (Figure 3).

Liver. The concentration of cholesterol in the liver of the intact or the hypophysectomized rat is not influenced by hemorrhage. This is illustrated in Table 1, where it can be seen that there is no difference between the level of cholesterol in the liver of the control or bled animals in either group.

It is a point of interest, however, that the liver of the hypophysectomized animal has a greater quantity of cholesterol per unit of tissue weight than that of the intact rat. The significance of this difference is at present not understood. It should be mentioned first that the values obtained in the hypophysectomized rats were quite variable and, second, that the livers of the hypophysectomized rat were 16 per

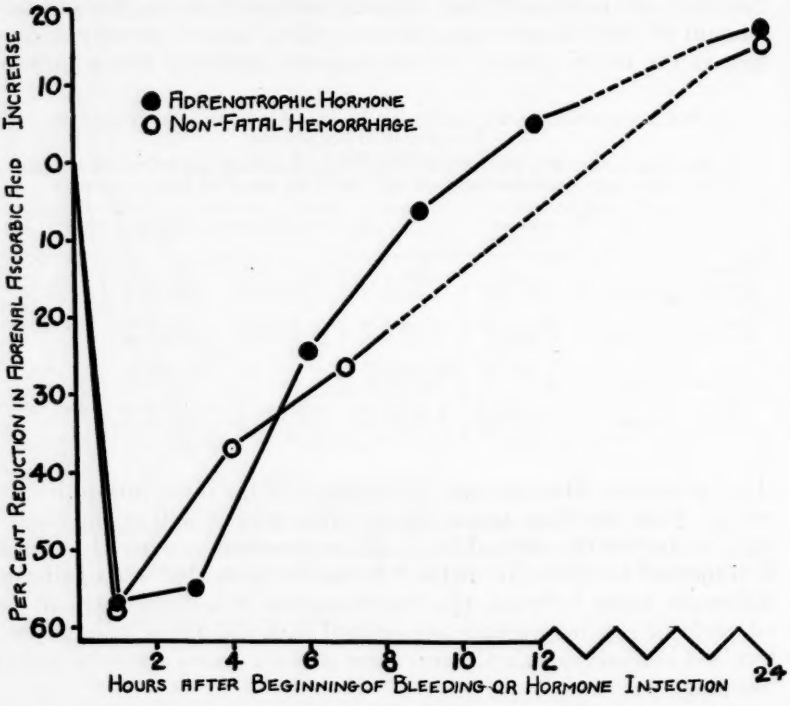

Fig. 2. Depletion of adrenal ascorbic acid following a non-fatal hemorrhage and following the administration of a single dose of adrenotrophic hormone.

cent smaller than those of the intact animals. When liver cholesterol values are calculated on the basis of body weight rather than liver weight, the difference between the two groups no longer exists.

The ascorbic acid concentration of the liver does change following bleeding. This substance is very definitely increased in concentration at the end of bleeding and remains elevated for a period of at least 24 hours in the intact rat (Table 2). The same phenomenon takes place in the absence of the pituitary, although here the observations were not extended beyond six hours. It would appear that the rat accelerates the synthesis of this vitamin in the liver in times of stress, but

whether this bears any relation to the well-known fact that the rat is more resistant to stress than the guinea pig cannot be decided.

Brain. Both the cholesterol (Table 1) and the ascorbic acid content of the brain (Table 2) are completely unaffected by simple hemorrhage or hypophysectomy.

Plasma. A uniform but slight decrease in the total cholesterol of the plasma of the intact rat took place following bleeding (Table 1).

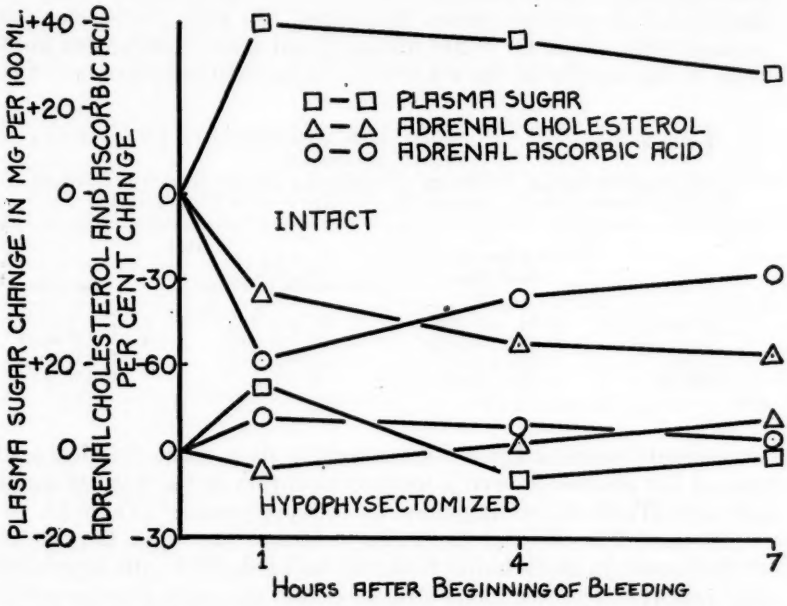

FIG. 3. Changes in plasma sugar, adrenal cholesterol and adrenal ascorbic acid of intact and hypophysectomized rats following a non-fatal hemorrhage.

The concentration of this substance returned to normal following recovery. The levels of cholesterol in the plasma of the hypophysectomized rats were quite variable, and the differences between the groups are not significant. The changes in the ascorbic acid content of the plasma are of some interest in light of reports that the urinary excretion of this vitamin is increased following the subjection of the organism to stress (Bowman and Muntwyler, 1936). The data are shown in Table 2. At the end of the bleeding period the level of ascorbic acid in the plasma of the intact rat is almost twice the initial control level. The concentration decreases from then on and returns to normal 24 hours after the hemorrhage. In the hypophysectomized animal there is no significant change in the average level of ascorbic acid in the plasma at the end of the bleeding period.

In summary, it would appear that a simple hemorrhage, from

which the animal can readily recover, markedly and specifically reduces the cholesterol and ascorbic acid content of the adrenal and that this effect is abolished by hypophysectomy.

Effects of a Fatal Hemorrhage Associated with Shock

The severity of the shock to which these animals were subjected was of such a character that replacement of a volume of blood sufficient to restore to the animal the quantity lost in hemorrhage was inadequate to prevent death. Following the final transfusion, the animals died within 63 to 244 minutes; and gross examination at the time of the sacrifice of the rat revealed a marked engorgement of the

TABLE 3. ADRENAL, LIVER, BRAIN AND PLASMA CHOLESTEROL FOLLOWING A FATAL HEMORRHAGE

Values expressed as mg. per 100 mg. of fresh tissue and mg. per 100 ml. of plasma. Each value represents the average and standard error of 5 to 7 animals.

	Controls (not bled)	Bled	
		Intermediate Stage	Terminal
Adrenal	5.84 ±0.314	2.58 ±0.414	1.53 ±0.140
Liver	0.391 ±0.0239	0.325 ±0.0093	0.330 ±0.0101
Brain	1.89 ±0.031	1.85 ±0.040	1.93 ±0.041
Plasma	55 ±2.6	43 ±1.8	42 ±3.5

abdominal viscera and gastro-intestinal bleeding, while chemical analyses of the plasma showed a marked elevation in the level of amino nitrogen (Table 5), accompanied by a hypoglycemia[3] (Table 5).

Adrenal. The effect of shock due to hemorrhage upon the content of cholesterol in the adrenal is shown in Table 3. At the intermediate stage (two to three hours after bleeding) the value is decreased to the same extent as in the case of the animals sacrificed 3 hours after a non-fatal hemorrhage (Table 1). It will be recalled, however, that in the case of the animals subjected to a non-fatal hemorrhage the concentration of cholesterol was not further decreased. In contrast, the concentration of this substance continued to diminish in hemorrhagic shock and at the time of respiratory collapse just preceding death (about 3½ hours after the end of bleeding) reached the low value of 1.5 mg. per 100 mg. of fresh tissue, a reduction of 70%. Three animals not included in Table 3 were subjected to hemorrhagic shock, and the period of survival was extended by a different technique to about eight hours after bleeding. The adrenal cholesterol reached a very low level in these animals, being 0.83, 0.55, and 0.38 mg. per 100 mg. of

[3] The level of plasma amino nitrogen in hypophysectomized rats subjected to a fatal hemorrhage increases to an extent quite comparable with that observed to take place in intact rats subjected to a fatal hemorrhage. The level of plasma amino nitrogen was 4.1 mg. per 100 ml. in control hypophysectomized rats and 11.8 mg. per 100 ml. in hypophysectomized rats dying in shock from hemorrhage.

fresh tissue. This testifies to the continuous and intense stimulation of the adrenal gland in hemorrhagic shock.

It is to be recalled that at the end of bleeding, in the case of the animals subjected to a non-fatal hemorrhage, the ascorbic acid content of the adrenal had declined to about one half its normal concentration in the gland. It is to be presumed that at the end of bleeding in the rats subjected to shock, the adrenal ascorbic acid had also reached this low level. The concentrations of this substance at the intermediate stage and at the point of final collapse show that the ascorbic acid level in the adrenal had not recovered to any extent previous to death (Table 4). Ascorbic acid is probably being used at a maximal rate

TABLE 4. ADRENAL, LIVER, BRAIN AND PLASMA ASCORBIC ACID FOLLOWING A FATAL HEMORRHAGE

Values expressed as mg. per 100 gm. of fresh tissue and mg. per 100 ml. of plasma. Each value represents the average and standard error of 5 to 7 animals.

	Controls (not bled)	Bled	
		Intermediate Stage	Terminal
Adrenal	403 ± 15.2	231 ± 11.6	205 ± 7.9
Liver	24.0 ± 1.10	20.5 ± 2.18	13.2 ± 0.99
Brain	39.6 ± 0.72	41.3 ± 0.69	38.9 ± 1.37
Plasma	0.51 ± 0.058	2.57 ± 0.128	3.0 ± 0.476

during this period, further demonstrating the intense stimulation of the adrenal in shock.

In contrast to the hyperglycemia found in rats subjected to a non-fatal hemorrhage, the blood glucose of those in shock declines (Table 5). There are probably several reasons for this: glycolysis in the peripheral tissues, impaired hepatic function and perhaps adrenal cortical insufficiency all acting as contributory causes.

Liver. The livers of animals in shock are from 6–8 per cent heavier than those of the controls. This increase is probably due to an abnormal accumulation of blood. Nevertheless, there is an unmistakable loss of ascorbic acid from the livers of animals in the terminal stage of shock with but little change in their content of cholesterol.

Brain. Neither the cholesterol nor ascorbic acid concentration of the brain (Tables 3 and 4) are altered at any time following a fatal hemorrhage.

Plasma. In spite of the fact that hemoconcentration is present in the animals in shock, the plasma cholesterol is definitely decreased. In contrast to this, there is a large increase in the plasma ascorbic acid, this substance being actually six times the normal concentration at the time of the final collapse.

This increase in plasma ascorbic acid is probably related to the loss of ascorbic acid from the liver (cf. above) and is a manifestation of the

damage to this organ that follows its exposure to a reduced rate of oxygen supply. The behavior of the liver ascorbic acid is in sharp contrast to the stability of this substance in the brain of the same animals (Table 4).

DISCUSSION

The experiments described in this paper demonstrate that a marked depletion of cholesterol and ascorbic acid occur in the adrenal following hemorrhage and add further support to the evidence that, following stresses of a diverse character, the functional activity of the adrenal cortex is increased.

It would appear that the adrenal cortex of the rat responds rapidly

TABLE 5. PLASMA, GLUCOSE AND AMINO NITROGEN FOLLOWING A FATAL HEMORRHAGE

Values expressed as mg. per 100 ml. of plasma.
Each value represents the average of 5 or 6 animals.

	Controls (not bled)	Bled	
		Intermediate Stage	Terminal
Period of time following end of bleeding (minutes)	—	166 (125–237)	208 (142–278)
Glucose	94.2 (84–97)	93.0 (83–102)	62.4 (54–68)
Amino Nitrogen	3.9 (3.2–4.2)	11.7 (7.5–16.1)	14.6 (11.3–16.9)

to the application of stress. In the case of hemorrhage of the degree reported in this paper, detectable lowering of the cholesterol and ascorbic acid occurs within an hour. In the caes of a hemorrhage from which the animal can spontaneously recover, the cholesterol and ascorbic acid content has returned to normal some twenty-four hours later. This may mean that either the activity of the gland declines as recovery proceeds, or that the rate of formation begins to exceed the rate of depletion, even though the latter may be continuing at an increased rate.

Since separate analysis of the cholesterol and ascorbic acid content of the adrenal cortex and medulla was not made in the rat, the question may arise as to the part played by the medulla in the observed changes. So far as ascorbic acid is concerned, there is good evidence that this is mainly concentrated in the cortex, and the magnitude of the changes observed leave little doubt that it is the content of this portion of the adrenal gland that is affected by stress. In the case of cholesterol the relative content of this substance in the adrenal cortex and medulla of the rat is not known, although examination of the

gland by staining technics or with the polarizing microscope indicate that here again the major concentration is in the cortex.

Other evidence, however, indicates that it is the cortical portion of the gland that is affected. Thus if rats with demedullated adrenals are exposed to cold, the adrenal cholesterol and ascorbic acid are depleted to the same degree as observed in animals with intact glands (Long and Fry, unpublished). In the guinea pig, it is possible separately to analyze the cortex and medulla. If these animals are injected with adrenotrophic hormone, then it is the cholesterol and ascorbic acid of the cortex that undergoes depletion to a degree comparable to that reported in this paper for the whole gland of the rat.

The high concentration and labile nature of cholesterol in the adrenal are of interest in connection with the possibility that cholesterol is the parent substance of the steroid hormones of this gland. A wide variety of external and internal stimuli which are known to cause an increased secretion of cortical hormone also deplete the adrenal cholesterol (Sayers *et al.*, 1944a). In the experiments described above, bleeding depleted the cholesterol content of the adrenal but not that of such organs as the liver or brain, an observation which strengthens the hypothesis that cholesterol is the precursor of the steroid hormone of the adrenal cortex.

Recently Bloch (1945) has advanced strong evidence that cholesterol is the parent substance of progesterone, a substance with a close chemical relationship to the active steroids of the adrenal cortex. This was shown by the conversion of cholesterol tagged with deuterium into pregnanediol, the excretory product of progesterone in man. It had previously been shown (Bloch, Berg and Rittenberg, 1943) that cholesterol tagged in a similar manner is converted into cholic acid, a substance whose basic structure is also closely related to the adrenal steroids.

The part played by ascorbic acid in the secretion or elaboration of the cortical hormones is not so evident. It might also be incorporated into the steroid structure of the hormones, but a recent finding of Lowenstein (personal communication) that the gland contains a highly active water soluble steroid, in which ascorbic acid is attached to ring D, indicates that the real importance of this substance may be in the formation of water soluble and hence easily diffusible hormones. This would be borne out by the observed fact that the depletion of adrenal ascorbic acid precedes that of the cholesterol in an activated gland.

It has been of some interest to understand the mechanism by means of which the adrenal cortex is stimulated to increased activity when the organism is subjected to stress. The progressive changes, both in the cholesterol and in the ascorbic acid content of the adrenal, which occur following a non-fatal hemorrhage, parallel in degree and in time those which occur following administration of adrenotrophic

hormone of the anterior pituitary. Furthermore, removal of the pituitary leaves the cholesterol and ascorbic acid content of the adrenal inert when the animal has suffered a non-fatal hemorrhage. This evidence leaves little doubt that it is the secretion of the anterior pituitary which stimulates the adrenal cortex to activity when the animal is exposed to stress. The mechanisms, humoral or nervous, which during stress stimulate the anterior pituitary are as yet unknown but obviously form an important field for future investigation.

The inert nature of two important metabolic constituents of the adrenal following hypophysectomy is in line with the present conception of the regulatory control of the pituitary over the adrenal cortex and is to be expected when one considers the inability of the hypophysectomized animal to survive abnormal conditions of the external or internal environment. However, the exact manner by which an increased adrenal cortical secretion enables an animal to withstand stress is unknown, although interesting, yet speculative, questions can be raised as to the association between this adrenal response and the increased protein catabolism that follows stress.

The dynamic changes in the ascorbic acid content of the adrenal which follow either a non-fatal hemorrhage or hemorrhagic shock are of interest in connection with the suggestions that have been made as to the possible use of this vitamin in the treatment of shock. The depletion of the vitamin may be of no consequence in the rat, an animal which can synthesize ascorbic acid, but in man and the guinea pig, in whom ascorbic acid is a dietary essential, cortical activity might be impaired when the available stores of this vitamin are rapidly used in the process of resistance of the organism to stress. It has also been suggested that maintenance of vitamin C stores is of importance in the resistance of the guinea pig and man to infection (Perla and Marmorston, 1937). Space does not permit review of the very extensive literature on the increased requirement of vitamin C by the human suffering from infectious diseases, toxic agents, and a wide variety of other pathological conditions, a common feature of which is adrenal cortical stimulation. The vitamin has been reported to have a beneficial effect in traumatic shock in the guinea pig (Ungar, 1942, Ungar, 1943, McDevitt, Duryee and Lowenstein, 1944) in hemorrhage in the cat (Stewart, Learmouth and Pollock, 1941) and in gravity shock in the rabbit (Locke, Locke and McIlroy, 1943). However, in a few experiments the administration of considerable quantities of the vitamin (20 mgs.) has had no beneficial effect on rats thrown into severe shock by hemorrhage.

It is probably best to state at the present time that, although the adrenal cortex is intensely activated by a variety of stresses which lead to shock, there is little evidence to indicate that the survival of such animals is limited by failure of adrenal cortical secretion. Other factors largely associated with the progressive tissue anoxia present

in this state appear to be of major importance. Among these are certain characteristic metabolic changes such as an elevation of plasma amino nitrogen, hypoglycemia and loss of ascorbic acid and other coenzymes from the liver, that are quite distinct from those associated with the normal response of the organism to a stress from which it can recover.

SUMMARY

Following a non-fatal hemorrhage both the cholesterol and ascorbic acid content of the adrenals of the rat are decreased. The fall in ascorbic acid precedes that of cholesterol, and it is restored more rapidly during the recovery period. The time relationships of these changes in the adrenal are similar to those following injection of adrenotrophic hormone into intact rats.

Hypophysectomy prevents these alterations in adrenal cholesterol and ascorbic acid after bleeding.

Shock induced in rats by hemorrhage is associated with marked depletion of adrenal cholesterol and ascorbic acid, and there is no tendency for recovery in the levels of these substances before death.

The cholesterol content of the liver and brain are unaffected by either fatal or non-fatal hemorrhage. The plasma cholesterol falls during shock due to hemorrhage.

The ascorbic acid of the liver rises after a non-fatal hemorrhage but is markedly decreased in shock due to hemorrhage. The plasma ascorbic acid rises slightly after non-fatal hemorrhage and is increased six fold in shock. The levels in the brain are unaffected.

It is suggested that the alterations in adrenal cholesterol and ascorbic acid are associated with the secretion of the cortical hormones and that they participate in their formation.

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STUDIES ON THE ROLE OF THE LIVER IN THE METABOLISM OF PROGESTERONE[1]

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MONTREAL

PROGESTERONE is inactivated in the body in the course of its metabolism (Zondek, 1939, 1941; Hamblen, Cuyler and Hirst, 1940). Pregnanediol-3(α),20(α) has been established as an end-product of progesterone metabolism in man (Venning and Browne, 1937; Westphal and Buxton, 1939) and in the rabbit (Heard, Bauld and Hoffman 1941; Westphal, 1942; Hoffman and Browne, 1942). Although pregnanediol-3(α), 20(α) is the only compound which has been shown conclusively to be a metabolite of progesterone, it accounts for less than 25% of the progesterone administered to these species. The fate of the remainder is not known.

Furthermore, little in known regarding the site or sites of metabolism of progesterone. Neither the ovary nor the uterus is essential for the conversion of progesterone into pregnanediol in the human female (Buxton, 1940; Venning and Browne, 1940; Jones and TeLinde, 1941) or in the female rabbit (Heard, Bauld and Hoffman, 1941). In the male rabbit this interconversion occurs in the absence of the testis (Hoffman and Browne, 1942). By a variety of experimental methods it has been shown that the liver of all laboratory animals so far studied plays an important role in the metabolism of oestrogens and androgens (for review of the literature see Biskind, 1942). The published reports regarding the role of the liver in the metabolism of progesterone are conflicting (for review of the literature see discussion). The present study was undertaken to evaluate these conflicting reports. The ability of the liver to inactivate progesterone was assessed by comparing the amount of progesterone which had to be administered by gavage to induce progestational proliferation of the endometrium of the normal rabbit, with the amount required in the partially hepatectomized rabbit.

EXPERIMENTAL PROCEDURE

The rabbit was used in this study because it is widely employed for the assay of progesterone, and because it is the only laboratory animal which is known to convert progesterone into pregnanediol-3(α), 20(α).

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Forty-nine immature female rabbits weighing between 700 and 900 gm. were used. Progesterone was administered, either subcutaneously or by stomach tube, to normal, sham-operated and partially hepatectomized rabbits which had been primed with α-estradiol.

Partial hepatectomy was performed by the method of Binet, Rouvillois and Bargeton (1939). The right lobe and approximately half of the left lobe of the liver were removed under nembutal anaesthesia. Immediately following the operation 10 to 15 cc. of a 10% solution of glucose in water were injected intravenously; twenty-four hours later 25 cc. of the glucose solution were given by gavage. As noted by Binet, Rouvillois and Bargeton the post-operative mortality rate was high.

In the sham-operated animals the abdomen was opened and the abdominal contents were exposed and manipulated in the same manner as in the animals which were partially hepatectomized.

The action of progesterone on the endometrium was determined by the method of McPhail (1934). The endometrium was primed by the subcutaneous administration of 5μg of α-estradiol on each of three alternate days. Forty-eight hours after the last injection, progesterone, in total amounts varying from 0.25 mg. to 200 mg., was administered subcutaneously or by stomach tube in equal, single, daily doses for five consecutive days. The daily subcutaneous dose was dissolved in 0.2 cc. of cotton seed oil; the daily oral dose was in solution in 2 cc. of cotton seed oil or in 2 cc. of a 10% solution of ethyl alcohol in propylene glycol. Twenty-four hours after the last administration of progesterone the animals were sacrificed, the uterus removed and studied histologically. The degree of progestational proliferation was expressed as 0 to ++++ according to the scale of McPhail.

The experimental animals were divided into six groups as follows:

Group I.—This group consisted of eleven normal rabbits. Each of six animals received a total dose of 0.25 mg. of progesterone, and each of the remaining rabbits a total dose of 0.5 mg., by subcutaneous injection.

Group II.—This group consisted of sixteen normal rabbits. All animals in this group were given progesterone by gavage in doses varying from 12.5 to 200 mg. for each animal as shown in the accompanying table.

Group III.—This group consisted of six animals on which a sham-operation was performed on the first day of progesterone treatment. Each animal received a total of 25 mg. of progesterone by gavage.

Group IV.—This group consisted of four animals on which a sham-operation was performed seventy-four hours after the first oestrogen injection. Progesterone treatment was begun seventy-two hours after the operation; each animal received a total of 75 mg. by gavage.

Group V.—This group consisted of six rabbits which were partially hepatectomized on the first day of progesterone treatment. Each of two animals received 12.5 mg. of progesterone by gavage; each of the remaining animals was given 25 mg. of progesterone by the same route.

Group VI.—This group consisted of six animals which were partially hepatectomized seventy-two hours after the first oestrogen injection. Progesterone treatment was begun seventy-two hours after the operation; a total of 25 mg. was given to each animal by gavage.

RESULTS

Since progesterone absorbed from the gastro-intestinal tract must

pass directly through the liver before reaching the uterus, it was thought that the effect of partial resection of the liver on progesterone inactivation could best be determined by administering the hormone by gavage. It is evident from the table that progesterone administered

THE EFFECT OF PROGESTERONE ON THE ESTROGEN-PRIMED ENDOMETRIUM OF NORMAL, SHAM-OPERATED AND PARTIALLY HEPATECTOMIZED RABBITS

Group	No. of animals	Experimental condition	Progesterone administered		Progestational proliferation of the endometrium[1]
			Total dose	Route	
			mg.		
I	6	Normal	0.25	Subcut.	++ (3); ++-+++(2); +++ (1)
	5	Normal	0.5	Subcut.	+++ (3); ++++(2)
II	2	Normal	12.5	Gavage	± (2)
	5	Normal	25	Gavage	0 (3); ± (2)
	2	Normal	50	Gavage	0 (2)
	2	Normal	100	Gavage	0 (1); ± (1)
	2	Normal	150	Gavage	0 (2)
	3	Normal	200	Gavage	++ (1); ++-+++ (2)
III	6	Sham-operation on First Day of Progesterone Treatment	25	Gavage	0 (4); ± (2)
IV	4	Sham-operation Three Days Prior to Progesterone Treatment	75	Gavage	0 (3); ± (1)
V	2	Partially Hepatectomized on First Day of Progesterone Treatment	12.5	Gavage	± (1); ++ (1)
	4		25	Gavage	+ (1); ++ (1); +++ (1); +++-++++ (1)
VI	6	Partially Hepatectomized Three Days Prior to Progesterone Treatment	25	Gavage	++ (3); ++-+++ (3)

[1] According to the scale of McPhail (1934); the numbers in brackets refer to the number of animals which showed the same response.

by stomach tube is much less effective than progesterone injected subcutaneously. Thus 200 mg. had to be given by gavage in order to obtain progestational proliferation comparable to that elicited by 0.25 mg. to 0.5 mg. of progesterone administered subcutaneously.

A dose of 25 mg. of progesterone administered to a partially hepatectomized rabbit by gavage, during the five-day period following operation, was effective in causing progestational transformation of the endometrium, while in the normal animal 200 mg. had to be administered to obtain a comparable progestational effect. Since the animal on which a sham-operation was performed did not respond to a dose of 75 mg. it seems likely that the increased sensitivity of the partially hepatectomized rabbit to progesterone is a consequence of the decrease in the mass of hepatic tissue and not a result of operative trauma. It is to be noted that a dose of 25 mg. of progesterone was

effective in causing progestational changes in the partially hepatectomized animal even when three days were allowed to elapse after the operation before progesterone treatment was begun. It would seem, then, that there is no important regeneration of functioning hepatic tissue in this time interval.

DISCUSSION

The data which have been presented demonstrate that progesterone administered by gavage is effective in producing progestational proliferation of the endometrium of the rabbit when administered in a dose which is 400 to 800 times greater than the effective subcutaneous dose. Previous investigators have noted the relative inactivity of orally administered progesterone and have proposed several theories in explanation. Engelhart (1932), Ehrhardt and Weigel (1933–34) and Fremery, Luchs and Tausk (1933) found that extracts of corpus luteum given orally in doses up to 12 "rabbit-units" were ineffective in inducing progestational proliferation of the endometrium of the rabbit. More recently Hohlweg and Inhoffen (1939) and Miescher and Gasche (1943) failed to obtain progestational changes in the endometrium of the rabbit by administering progesterone orally in amounts up to 60 mg.; with 100 mg. the latter authors obtained a slight progestational effect. Portman (1935) obtained a slight localized decidual transformation with 25 "rabbit-units" of corpus luteum extract administered orally to the rabbit. Although a more definite response was obtained by this author with 50 "rabbit-units" it was not as marked as that elicited by 1 "rabbit-unit" of the extract administered subcutaneously. Soderwall (1940) using sexual receptivity as a criterion, found that in the estrogen-treated spayed female guinea pig the effective oral dose of progesterone was about twenty times greater than the subcutaneous dose.

Engelhart (1932) attributed the inactivity of orally administered corpus luteum extract to destruction of the hormone in the gastrointestinal tract as a result of the action of digestive enzymes. Destruction of progesterone in, or its poor absorption from, the gastrointestinal tract does not appear to be a likely explanation of the ineffectiveness of orally administered progesterone, since as much pregnanediol appears in the urine of rabbits following the administration of progesterone by gavage as when the same dose is administered subcutaneously (Hoffman, 1944).

The relative ineffectiveness of orally administered progesterone, as contrasted with subcutaneously injected progesterone, might in part be explained by the assumption that progesterone is more rapidly absorbed from the gastro-intestinal tract, and consequently more rapidly inactivated and excreted. Although there is no direct evidence to support this hypothesis, studies on the effect of the route of ad-

ministration of another steroid, dehydroisoandrosterone, on the excretion of its metabolites provide some support. It was found in the male rabbit that the 17-ketosteroids derived from the metabolism of dehydroisoandrosterone were more rapidly excreted in the urine when dehydroisoandrosterone was administered by stomach tube, than when it was injected subcutaneously (Hoffman, 1944).

The foregoing experiments have shown that in the partially hepatectomized rabbit the intragastric dose of progesterone required to induce progestational changes in the endometrium is about one-eighth the dose required in the normal animal. It would appear then that the liver is an important site of progesterone inactivation. Since progesterone absorbed from the gastrointestinal tract must pass directly through the liver before reaching the uterus it seeems likely that the reduced effectiveness of orally administered progesterone is largely the result of its inactivation in the liver.

There is some disagreement in the literature concerning the role of the liver in the metabolism of progesterone. Zondek (1939, 1941) found that although progesterone was inactivated in the body of the rat, "in vitro" experiments indicated that the liver is not involved in the inactivation process. Selye (1941) and Selye and Stone (1944), on the other hand, inferred that the liver of the rat is a site of inactivation of progesterone since the anesthetic action of orally administered progesterone is greatly enhanced by partial hepatectomy. Dosne (1944) also concluded that the liver inactivates progesterone, because she found that pellets of progesterone implanted in the spleen of the estrogen-treated spayed female guinea pig were less antifibromatogenic than pellets of progesterone implanted subcutaneously. In the rabbit, Mussio-Fournier, Morató-Manaro and Albrieux (1937–1940) found that progesterone injected into the liver or spleen was less effective in inducing progestational changes in the endometrium than progesterone injected subcutaneously, and hence they concluded that the liver was responsible for the destruction of progesterone. Fels and Monacó (1941), however, were unable to confirm these latter experiments and expressed the view that the liver does not inactivate progesterone. Engel (1944) found that under conditions which are known to inactivate estrogens, pulp prepared from the liver of a nonpregnant mare did not inactivate progesterone "in vitro." Kochakian, Haskins and Bruce (1944), compared the progestational response obtained by implanting pellets of progesterone in the mesentery, in the kidney, in the muscle and under the skin, and concluded that the liver is the chief site of inactivation of progesterone in the rabbit.

From a survey of the literature and from the experiments herein described it seems justifiable to conclude that "in vivo" the liver is an important site of progesterone metabolism. The published reports indicate that "in vitro" liver tissue does not inactivate progesterone.

SUMMARY

Progesterone administered by stomach tube was found to be less effective in inducing progestational transformation of the endometrium of the normal rabbit than when administered subcutaneously. In the normal estrogen-primed immature rabbit 200 mg. of progesterone had to be administered by gavage to obtain a progestational response comparable to that elicited by 0.25 to 0.5 mg. administered subcutaneously. In the partially hepatectomized rabbit, on the other hand, comparable progestational changes were obtained by the administration of 25 mg. of progesterone by gavage. This striking decrease in the intragastric dose required to cause progestational proliferation is interpreted as indicating that the liver plays an important role in the inactivation of progesterone in the rabbit. The relative inactivity of progesterone, administered by gavage, in the normal rabbit may, therefore, be explained in part by the fact that progesterone administered by this route must pass directly through the liver before reaching the endometrium.

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VICARIOUS METABOLIC RESPONSE[1]

The Influence of Blood from Thyroxinized Animals on the Oxygen Consumption of Surviving Tissue[2]

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NEW HAVEN

REPEATED STUDY has shown that the blood iodine in hyperthyroidism tends to be abnormally high (Salter, 1944). Furthermore the "organic" or "hormonal" iodine accounts for most of the increase (Bassett, Coons and Salter, 1941). This finding suggests that the circulation contains an excess of a physiologically active iodine compound. It is the purpose of this communication to show that such blood can increase the respiration of surviving tissues.

The time factor looms large in experimental attempts to demonstrate such stimulation of metabolism in vitro. Thus Davis and Hastings (1936), using the heart of *Limulus polyphemus*, obtained an elevation of respiration only after 10 to 15 hours' exposure to thyroxine *in vitro*. Hopping Scott (1935), however, reported in some instances a prompt increase in oxygen consumption by the nucleated red cells of alligator blood when thyroxine was added *in vitro*. In the present communication emphasis will be laid upon the prompt respiratory response of mammalian tissues (when a certain technical procedure is followed) to incubation with blood from hyperthyroid animals.

The experiments reported herewith were performed with liver tissue and with implanted sarcoma 180 grown in pure-strain mice. These experiments were designed to answer the following question: Will surviving tissues from normal animals respond by increased respiration when treated with blood from hyperthyroid animals?

This experimental program was an extension of the technique of Davis and Hastings (1936), who demonstrated an increased consumption of oxygen by the heart of *Limulus*, as already mentioned, during prolonged incubation with thyroxine. We used blood from hyperthyroid animals as an incubating medium for the surviving tis-

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sues after experiments had failed to demonstrate any change in metabolism when thyroxine was added to normal blood or to Ringer's solution. The tentative supposition was that the actual circulating hormone may exist in a more effective state than thyroxine, i.e., as a modified thyroxine derivative. Tumor was chosen as one of the tissues because of its ability to survive *in vitro* for considerable period (Warburg, 1931).

EXPERIMENTAL PROCEDURES

The data to be presented consist essentially of comparative measurements of the oxygen consumption of surviving tissue slices which had been previously incubated in either normal or hyperthyroid blood. A simultaneous comparison also was made, in some of the experiments, with the initial oxygen consumption of the tissue immediately before incubation.

The "hyperthyroid blood" used as the incubating medium was obtained from mice or guinea pigs previously injected subcutaneously with thyroxine dissolved in 0.001 N sodium hydroxide. The control animals received injections of dilute alkali. The dose of thyroxine given was 0.04 mg. per 25-gram mouse, or 0.4 mg. per 250 gram guinea pig. This dose was repeated daily for from five to twelve days for mice and from two to five days for guinea pigs. The animal blood was obtained by guillotining. Clotting was prevented by adding sufficient heparin to make a concentration of 0.2 per cent.

The respiration of the mouse liver and of the sarcoma 180 was measured by the technique developed by Warburg (1931), using the single vessel method.

EXPERIMENTS WITH TUMOR

In the experiments with sarcoma 180 the following procedure was used. Immediately following the bleeding of the normal and the hyperthyroid animals, sarcoma 180 from black mice of the C-57 strain was sliced into Ringer-phosphate solution containing glucose at room temperature. Occasional tests showed the thickness of the slices to be well within 0.5 mm. Representative slices were placed in the normal and hyperthyroid blood for incubation. Concomitantly, other slices were placed in Warburg vessels containing Ringer-phosphate-glucose solution, for the determination of the initial oxygen consumption in the experiments in which this determination was made. After incubation for a given time, the slices in the blood were removed and their oxygen consumption likewise determined in Ringer-phosphate-glucose solution. The details of the procedure and the methods of calculation are brought out in the following protocol.

Protocol I. Experiment 62

The mice used in this experiment had received daily injections of 0.03 mg. thyroxine in 0.5 cc. of 0.001 normal sodium hydroxide or of alkali alone for 11 days. One alkali-injected control (A) and one thy-

roxine-injected (T) mouse were sacrificed. The blood was collected from the neck at 11:45 A.M. into 50 cc. Erlenmeyer flasks containing 5 mg. heparin. Next, a mouse sarcoma 180 was excised and sliced into Ringer-phosphate-glucose solution at 27° C. Companion slices were then placed in the flasks containing the blood. By twelve oclock the flasks were filled with a gas mixture of carbon dioxide (5 per cent) and oxygen (95 per cent). At the same time other slices were placed in Warburg vessels for determination of the initial Qo_2. Check determinations gave initial Qo_2 values of 7.6 and 7.3.

The flasks were allowed to stand for four hours at 27° C. (room temperature) with gentle agitation at intervals of from 15 to 30 minutes. The slices were then transferred to vessels containing 2.0 cc. of Ringer-phosphate-glucose and also 0.2 cc. of the blood, "A" or "T," in which they had been standing. In the inset of the vessel, 0.2 cc. of 10 per cent sodium hydroxide was placed to absorb carbon dioxide. The vessels were filled with oxygen and placed in a thermostat at 37.5 ± 0.02° C. They were shaken 50 times a minute. After a 10-minute period for equilibration, readings were begun and continued at 15-minute intervals for 1 hour. For each blood, two vessels containing tumor were studied; a third vessel was observed as a blank.

In calculating the results, the pressure change, h, was multiplied by the vessel constant to give the oxygen consumption, x, in cubic millimeters. At each time interval the blank was subtracted. The corrected value, x', was divided by the dry weight of the tissue, expressed in milligrams, y. This value was obtained after heating the tissues in an oven at 100° C. for 2 hours following the measurement of oxygen consumption. The oxygen consumption per milligram of dry weight per hour, or the Qo_2, was found to be 6.5 and 6.4 in the "A" blood and 8.3 and 8.1 in the "T" blood. The elevation in oxygen consumption produced by the "T" blood was considered to be $(8.3-6.5)/6.5$, or 28 per cent, above the controls. This increment was only $(8.3-7.5)/7.5$, or 11 per cent, above the absolute value of the *initial level;* but because this control value declines with time, such a calculation gives only a minimal value. The latter value, therefore, is not considered to be as reliable as the former. The data for this experiment are recorded in Table 1.

Data on Oxygen Consumption

Exploratory experiments were performed with tumor tissue to determine the conditions necessary for a clear demonstration of the vicarious metabolic response. Among these circumstances were the effects (a) of duration of incubation and (b) of the temperature at which incubation was carried out. These results are recorded in Table 2, which shows that at about 20° C. the effect is maximal. Incubation for two hours at 20° C. gave a larger response than incubation for four hours.

TABLE 1. REPRESENTATIVE PROTOCOL: EXPERIMENT 62. DETERMINATION OF OXYGEN CONSUMPTION IN RINGER-PHOSPHATE-GLUCOSE OF SARCOMA 180 TUMOR SLICES FOLLOWING INCUBATION FOR FOUR HOURS IN BLOOD OBTAINED FROM A CONTROL MOUSE AND FROM A MOUSE MADE HYPERTHYROID WITH THYROXINE

Vessels filled with oxygen; 0.2 cc. 10% NaOH in inset. Temperature 37.5° C.

Control Tumor after Four Hours in Control Blood

Medium: 2.00 cc. Ringer-phosphate-glucose + 0.2 cc. blood from control mouse

Vessel no.:	6		4H				14			
Volume in cc.:	13.42		16.96				20.18			
Vessel constant in cc.:	0.97		1.28				1.56			
Dry wt. of tissue, mg.:	blank		7.4				6.3			
Time (min.)	h (mm.)	x (cu.) (mm.)	h (mm.)	x (Cu.) (mm.)	x'	$\frac{x'}{y}$	h	x	x'	$\frac{x'}{y}$
15	3	3	13	17	14	1.9	9	14	11	1.7
30	4	4	22	28	24	3.2	15	23	19	3.0
45	6	6	34	44	38	5.1	23	36	30	4.8
60	7	7	43	55	48	6.5	30	47	40	6.4

Control Tumor after Four Hours in Hyperthyroid Blood

Medium: Ringer-phosphate-glucose, 2.0 cc., plus 0.2 cc. blood from thyroxine-injected mouse.

Vessel no.:	5		3				4			
Volume in cc.:	12.96		11.64				11.49 cc.			
Vessel constant:	0.93		0.82				0.80			
Dry wt. of tissue, mg.:	blank		4.6				4.2			
Time (Min.)	h (mm.)	x (cu.) (mm.)	h (mm.)	x (cu.) (mm.)	x'	$\frac{x'}{y}$	h	x	x'	$\frac{x'}{y}$
15	2	2	14	11	9	2.0	14	11	9	2.1
30	4	4	29	24	20	4.4	27	22	18	4.3
45	6	6	42	34	28	6.1	39	31	25	6.0
60	9	8	56	46	38	8.3	52	42	34	8.1

In Tables 3, 4, and 5 are given the results of three independent series of determinations of the oxygen consumption of tumor slices from normal animals. The test slices were incubated with blood from animals made hyperthyroid with thyroxine. There was one long series (Series A, Table 3) and two additional shorter ones (Series B, Table 4 and Series C, Table 5). These were carried out in 1939 from March to November, in 1938 in October and November, and 1939 in April and May, respectively. The data have been subjected to statistical analysis by applying the theory of probability. The method is that outlined by R. A. Fisher (1936). We are indebted to Miss Barbara L. Bartels for many of the computations included in this paper.

TABLE 2. INFLUENCE OF TEMPERATURE AND TIME OF INCUBATION ON THE RESPONSE OF TUMOR TO HYPERTHYROID BLOOD

Exp.	Initial Q_{O_2}				Q_{O_2} of tumor after incubation					
	Livers of Blood Donors			Tumor	2 Hours			4 Hours		
Exp.	A	T	T/A		A	T	T/A	A	T	T/A
					8°					
430	9.2 8.2	13.2 11.7	1.43	7.1 8.0	9.3 8.3	9.2 8.8	0.99 1.06	10.3 8.2	9.7 8.1	0.94 0.99
443	9.9 10.7	11.3 12.2	1.14	6.4	8.8 8.4	9.3 8.2	1.06 0.98	7.5	8.1 7.5	1.08
446	12.0 11.2	12.8 11.4	1.04	6.8 7.1	7.4 8.3	7.9 7.9	1.07 0.95	9.1	8.3	0.91
447	10.4 9.9	12.1 12.1	1.19	6.8 6.3	8.1 8.0	7.9 7.2	0.98 0.90	8.0 7.4	8.5 7.4	1.06 1.00
Av.			1.20	6.9	8.3	8.3	1.00	7.9	8.2	0.99
Av. of $\frac{\text{final}}{\text{initial}}$					1.21	1.21		1.21	1.19	
					15°					
432	10.4 9.8	10.5 11.6	1.09	7.7 7.3	7.7 7.5	7.7 7.3	1.00 0.97	7.4 6.9	7.6 6.8	1.03 0.99
448	10.0 9.5	12.0 11.3	1.19	6.9 7.5	8.2 7.4	7.6 7.4	0.93 1.00	7.4 7.2	7.3 6.8	0.99 0.94
449	10.9 10.1	12.0 12.0	1.14	7.7	8.4 8.0	8.2 8.0	0.98 1.00	9.5 8.4	10.0 8.5	1.05 1.01
450	9.9 10.4	11.8 13.9	1.27	7.6	8.0 8.4	7.4 8.0	0.93 0.95	8.1 6.6	8.4 6.7	1.04 1.01
Av.			1.17	7.4	7.9	7.7	0.97	7.7	7.7	1.01
Av. of $\frac{\text{final}}{\text{initial}}$					1.06	1.03		1.03	1.03	
					20°					
416	10.6 9.2	13.8 14.5	1.43	6.1 5.9	5.0 3.8	6.8 6.3	1.36 1.66	5.6 5.6	6.3 5.6	1.12 1.00
419	10.6 10.2	13.1 12.2	1.22	7.6 7.3	8.7	8.4	0.97	6.7 6.8	8.1 8.3	1.21 1.22
420	8.6 8.6	10.1 13.2	1.35	7.3 6.5	7.7	7.1	0.92	7.2	7.9	1.10
421	7.4 9.2	8.8 10.7	1.17	5.9 6.3	6.8	8.2	1.21	7.2	7.4	1.03
422	9.4 6.1	12.3 10.8	1.53	7.0 6.6	7.6	8.9	1.17	8.4	7.2	0.86
424	10.2 9.4	12.3 11.0	1.19	6.2 6.4	8.3	10.0	1.20	8.0	8.2	1.02

TABLE 2—*Continued*

Exp.	Initial Qo_2				Qo_2 of tumor after incubation					
	Livers of Blood Donors			Tumor	2 Hours			4 Hours		
Exp.	A	T	T/A		A	T	T/A	A	T	T/A
				20°						
425	8.8 7.6	11.7 10.3	1.34	5.9 5.6	7.3	7.3	1.00	7.6	7	1.01
431	8.9 8.0	9.3 10.3	1.16	6.6 5.5	5.9	8.1	1.37	7.5	9.6	1.28
Av.			1.29	6.4	6.8	7.9	1.21	7.1	7.6	1.08
Av. of $\frac{\text{final}}{\text{initial}}$					1.06	1.25		1.09	1.18	
				25°						
437	10.6 9.9	11.2 12.1	1.14	7.5 7.8	7.1 6.6	9.7 7.8	1.37 1.18	7.0 5.6	6 9 6.7	0.99 1.20
436	11.2 9.4	12.9 13.5	1.28	7.7 7.0	8.8 8.8	8.2 9.3	0.93 1.06	7.7 7.2	7.9 7.0	1.03 0.97
435	10.2 10.4	12.9 12.6	1.24	6.1 6.6	6.5	7.4	1.14	5.7	6.2	1.10
Av.			1.22	7.1	7.6	8.5	1.12	6.6	6.9	1.04
Av. of $\frac{\text{final}}{\text{initial}}$					1.02	1.16		.91	.95	
				30°						
427	10.1 8.0	11.0 11.9	1.26	6.9 6.6	7.1 6.7	6.7 6.9	0.94 1.03	7.5 6.2	6.6 5.6	0.88 0.91
426	9.7 8.0	8.9 10.3	1.08	7.2 7.2	6.7 6.7	7.5 7.8	1.12 1.16	6.8 6.0	7.0 6.9	1.03 1.15
428	7.7 9.5	12.3 11.7	1.40	7.6 6.9	6.6	6.7	1.01	6.4	6.8	1.06
429	9.7 9.4	12.1 11.2	1.22	7.1	7.1 7.1	7.1 6.8	1.00 0.96	7.1 5.8	7.7 5.6	1.08 0.97
Av.			1.24	7.1	6.9	7.1	0.98	6.4	6.6	1.03
Av. of $\frac{\text{final}}{\text{initial}}$				(1.0)	0.97	1.00		0.92	0.93	
				37.5°						
431	8.9 8.0	9.3 10.3	1.16	6.6 5.5	5.2	5.9	1.13	6.7	5.7	0.85

TABLE 2—*Continued*

Exp.	Initial Qo_2				Qo_2 of tumor after incubation					
	Livers of Blood Donors			Tumor	2 Hours			4 Hours		
Exp.	A	T	T/A		A	T	T/A	A	T	T/A
440	9.4 10.2	11.2 11.3	1.17	7.7	6.2 5.4	6.0 5.7	0.97 1.06	5.7 5.6	5.1 4.6	0.89 0.82
439	7.9 8.8	11.9 12.5	1.46	7.2 6.7	5.6	5.7 5.5	1.00			
411	7.4 7.7	12.7 14.1	1.77	7.2	6.3	6.9	1.09			
441	8.9 10.1	12.5 12.4	1.31	5.6	3.8	4.8	1.26	3.2 3.0	3.7 2.8	1.16 0.93
442	10.8 8.7	11.8 11.2	1.18	7.8 7.0	5.8	6.6	1.14	5.0 4.9	4.9 4.0	0.98 0.82
Av.			1.34	6.7	5.5	5.9	1.09	4.9	4.4	0.90
Av. of $\frac{\text{final}}{\text{initial}}$				(1.0)	0.79	0.85		0.71	0.66	

Statistical P("T" vs. "A" paired) <0.05.

All series show essentially the same average result, namely, that the Qo_2 for the tissue incubated with hyperthyroid blood was 20 or 21 per cent higher than that of the controls. In Series A, Table 3, although there is a wide range of variation, comparison of the mean Qo_2 for the tumor tissue incubated in hyperthyroid blood with that of the tissue incubated in control blood showed highly significant differences; $P < .001$. When the differences ΔQo_2) between the "T" value and the "A" value for each individual experiment were analyzed, such differences could have occurred by chance only one in 100 times ($P < 0.01$). When the data were analyzed to test the significance of the regression of "T" on "A," that is, to see whether an increase in the "A" value was correlated with an increase in the "T" value, a significant correlation was found ($P < 0.001$).

The shorter series B, Table 4, consisting of only 10 experiments showed a somewhat less significant difference between the mean Qo_2's of the hyperthyroid and control values ($P < 0.05$). Analysis of the differences between "T" and "A" for each individual experiment (ΔQo_2) showed these differences to be significant ($P < 0.05$).

Series C, Table 5, in addition to corroborating the results obtained in the first two series, demonstrates the following points. The vicarious response found after two hours may disappear if one incubates for four hours. At two hours, comparison of the initial oxygen consumption of the tumor slices before incubation with the oxygen consumption following incubation in "T" blood showed a significant increase in

TABLE 3. OXYGEN CONSUMPTION IN RINGER-PHOSPHATE-GLUCOSE OF SARCOMA 180 TISSUE SLICES FOLLOWING INCUBATION FOR TWO HOURS IN BLOOD FROM ALKALI-INJECTED CONTROL MICE AND IN BLOOD FROM THYROXINE-INJECTED MICE

No.	Exp.	Q_{O_2} after blood from animals injected with		Difference (=T−A)	Percentage difference
		Alkali (controls)	Thyroxine		
1	76	6.8	6.5	−0.3	−5
2	78	8.9	8.6	−0.3	−4
3	79	6.9	7.9	1.0	
4		6.8	7.6	0.8	
5		7.7	8.2	0.5	11
6		7.5	8.3	0.8	
7	80	5.9	8.3	2.4	23
8		7.4	8.1	0.7	
9	81	7.2	7.9	0.7	20
10		6.8	8.9	2.1	
11	86	6.9	7.3	0.4	6
12		6.7	7.1	0.4	
13	88	7.0	7.7	0.7	16
14		6.8	8.3	1.5	
15	91	6.0	5.5	−0.5	3
16		5.5	6.5	1.0	
17	92	6.6	8.7	2.1	19
18		6.9	7.3	0.4	
19	97	6.4	6.1	−0.3	5
20		5.9	6.9	1.0	
21	98	5.2	7.3	2.1	30
22		5.3	6.3	1.2	
23	99	5.7	7.3	1.6	26
24		5.7	7.0	1.3	
25	54	8.5	9.7	1.2	14
26	55	7.1	8.3	1.2	17
27	62	6.5	8.3	1.8	30
28		6.3	8.3	2.0	
29	66	7.6	7.5	−0.1	3
30		6.7	7.2	0.5	
31	67	4.5	7.7	3.2	55
32		5.5	7.8	2.3	
33	69	5.5	7.1	1.6	
34		5.3	7.4	2.1	39
35		5.3	7.6	1.8	
36		5.0	8.0	3.0	
37	70	5.8	7.3	1.5	
38		5.4	7.0	1.6	
39		5.4	8.7	3.3	33
40		6.0	7.1	1.1	
41	71	6.9	8.2	1.3	
42		5.0	7.8	2.8	32
43		5.9	7.8	1.9	
44		4.9	7.6	2.7	
45	72	7.4	8.6	1.2	
46		6.0	8.3	2.3	25
47		7.0	8.6	1.6	
48	68	5.8	6.9	1.1	8
49		7.4	7.3	−0.1	
50	118	6.4	8.4	2.0	31
51	120	5.6	5.9	0.3	5
52	122	6.6	9.8	3.2	48
53	126	5.4	6.1	0.7	4
54			5.1	−0.3	
55	146	8.0	7.3	−0.7	−9
56	155	8.1	12.3	4.2	52
Average		6.39	7.70	1.31	20.5
Standard Deviation		0.96	0.75	1.04	

Statistical P("T" vs. "A") <0.001. $P(\Delta Q_{O_2}) < 0.01$.
Regression of "T" on "A," slope $b = 0.38$, $P < 0.001$.

TABLE 4. OXYGEN CONSUMPTION IN RINGER-PHOSPHATE-GLUCOSE OF SARCOMA 180 TUMOR SLICES FOLLOWING INCUBATION FOR TWO HOURS IN BLOOD FROM ALKALI-INJECTED CONTROL MICE AND IN BLOOD FROM THYROXINE-INJECTED MICE

No.	Exp.	Q_{O_2} after blood from animals injected with		Difference (=T−A)	Percentage difference
		Alkali (controls)	Thyroxine		
1	323	7.5	8.5	1.0	13
2	324	9.4	10.0	0.6	6
3	333	2.5	6.0	3.5	140
4	335	6.8	8.3	1.5	22
5	340	8.2	9.5	1.3	16
6	341	8.7	8.7	0.0	0
7	342	7.6	9.1	1.5	20
8	345	8.3	9.6	1.3	16
9	347	7.6	7.5	−0.1	−2
10	351	4.9	8.6	3.7	76
Average		7.15	8.58	1.43	20

Statistical P("T" vs. "A") <0.05. $P(\Delta Q_{O_2}) < 0.05$.

Q_{O_2} as a result of the incubation ($P<0.01$). When the tumor slices were incubated in "A" blood, the increase in Q_{O_2} was not significant ($P>0.6$). This finding indicates that incubation of tumor slices in control blood has no significant effect on their subsequent oxygen consumption, at least when the incubation is only for two hours.

It will be noted in Series C, that the oxygen consumption of the tumor slices following incubation in the control and hyperthyroid blood was determined in a medium containing 2.0 cc. of Ringer-phosphate-glucose plus 0.2 cc. of the blood in which they had been incubated. It was thought that the addition of the blood to the medium when determining the final Q_{O_2} might increase the metabolic response to the hyperthyroid blood. Careful examination of this point, however, showed that the addition of 10% blood to the Ringer-phosphate-glucose medium had no significant effect in either exaggerating or minimizing the difference. The data are presented in Table 6. The increase in the Q_{O_2} of the tumor tissue brought about by incubation in "T" blood was not altered significantly by the addition of 10% blood to the Ringer-phosphate-glucose medium ($P>0.4$).

pH. The presence of 10 per cent blood in the Warburg vessel during the metabolism test did not alter the pH of the medium to any significant extent as these figures for experiment 437 show. The pH was determined by glass electrode following the removal of the tissue at the end of the metabolism measurement.

Tissue	Added Blood	pH
From A blood	A	6.95
From A blood	A	6.95
Blank	A	7.20
From T blood	T	6.90
From T blood	T	7.00
Blank	T	7.15
Before addition of blood or tissue	RPG	7.20

TABLE 5. THE RIGHT SIDE OF THE TABLE GIVES THE OXYGEN CONSUMPTION OF SARCOMA 180 TUMOR SLICES FOLLOWING INCUBATION FOR TWO HOURS AND FOUR HOURS IN BLOOD FROM ALKALI-INJECTED CONTROL MICE AND IN BLOOD FROM THYROXINE-INJECTED MICE. DETERMINATIONS WERE MADE IN RINGER-PHOSPHATE-GLUCOSE CONTAINING 10% OF THE BLOOD IN WHICH THE RESPECTIVE SLICES WERE INCUBATED. THE LEFT SIDE OF THE TABLE GIVES THE OXYGEN CONSUMPTION OF THE LIVERS FROM THE RESPECTIVE CONTROL (ALKALI-INJECTED) AND THYROXINIZED DONOR MICE. THE INITIAL OXYGEN CONSUMPTION OF THE TUMOR IN RINGER-PHOSPHATE-GLUCOSE BEFORE INCUBATION IS ALSO GIVEN

Initial Q_{O_2} in RPG					Q_{O_2} of Tumor in 10% Blood after Incubation at 20°					
Liver				Tumor	2 Hours			4 Hours		
Exp.	A	T	T/A		A	T	T/A	A	T	T/A
414	8.9 7.2	9.0 10.8	1.23	5.3 6.2	4.3	6.0	1.40	6.9	6.8	0.99
415	11.8 9.7	11.5 13.8	1.17	7.6 6.8	6.2	7.4	1.19	8.5	6.9	0.81
416	10.6 9.2	13.8 14.5	1.43	6.1 5.9	5.0 3.8	6.8 6.3	1.36 1.66	5.6 5.6	6.3 5.6	1.12 1.00
419	10.6 10.2	13.1 12.2	1.22	7.6 7.3	8.7	8.5	0.98	6.7 6.8	8.1 8.3	1.21 1.22
420	8.6 8.6	10.1 13.2	1.35	7.3 6.5	7.7	7.1	0.92	7.2	7.9	1.10
421	7.4 9.2	8.8 10.7	1.17	5.9 6.3	6.8	8.2	1.21	7.2	7.4	1.03
422	9.4 6.1	12.3 10.8	1.49	7.0 6.6	7.6	8.9	1.17	8.4	7.2	0.86
424	10.2 9.4	12.3 11.0	1.19	6.2 6.4	8.3	10.0	1.20	8.0	8.2	1.02
425	8.8 7.6	11.7 10.3	1.34	5.9 5.6	7.3	7.3	1.00	7.6	7.7	1.01
Average			1.29				1.21			1.03
Av. increase = +29% Statistical $P_\Delta < 0.01$					Av. incr. = +21% $P\Delta < 0.01$			Av. incr. = +3%		

P (initial vs. "A") < 0.6. P (initial vs "T") < 0.01

EXPERIMENTS WITH LIVER TISSUE

The foregoing experiments were extended to surviving liver tissue with the modification that the metabolism was measured at once in the appropriate blood plasma without preliminary incubation. A typical protocol follows:

Protocol II Experiment 178

Guinea-pig Plasma–Mouse Liver

Thyroxine was injected under the skin of guinea pigs for four days in doses of 0.385 mg. per day dissolved in 0.5 cc. of 0.001 normal sodium hydroxide. Alkali alone was injected into the control guinea

pigs. The effect of the injections upon the animals' weight was as follows:

Date		1/12	1/13	1/14	1/15	1/16
Weight in grams	A	229	219	231	219	219
	T	249	218	211	201	206

Blood was taken from the neck into beakers containing 0.8 cc. of heparin solution (yielding 50 mg. of heparin per cubic centimeter). The "T" pig, killed at 4:25 P.M., yielded 9 cc. of blood; the "A" pig, killed at 4:30 P.M., yielded 11 cc. of blood. Each blood sample was then centrifuged and the plasma removed.

The test liver was removed from a normally fed mouse at 4:35 P.M. and sliced into horse serum previously warmed to 37.5° C. Two

TABLE 6. Q_{O_2} OF SARCOMA 180 AFTER INCUBATION WITH A AND T BLOOD: EFFECT OF 10% BLOOD IN VESSEL ON T/A RATIO

Measurements in RPG					Measurements in RPG plus 10% Blood		
Exp.	Hours of incubation	A	T	T/A	A	T	T/A
71	4	6.9	8.2	1.19	5.2	7.7	1.48
72	4	7.4	8.6	1.16	6.0	8.5	1.42
79	4	6.9	7.8	1.16	7.6	8.3	1.09
81	4	7.0	8.4	1.20			
397	4	6.7	6.5	0.97	7.0	7.5	1.07
414	2	7.1	7.2	1.01	4.3	6.0	1.39
414	4	5.3	5.6	1.06	6.9	6.8	0.99
419	2	6.5	7.6	1.17	8.7	8.5	0.97
420	2	6.6	6.8	1.03	7.7	7.9	1.09
420	4	6.9	8.4	1.22	7.2	7.9	1.10
421	2	5.8	6.1	1.05	6.8	8.2	1.21
421	4	4.8	4.5	0.94	7.2	7.4	1.03
422	2	6.4	7.0	1.09	7.6	8.9	1.17
422	4	4.8	5.9	1.23	8.4	7.2	0.86
424	2	6.6	7.0	1.09	8.3	10.0	1.20
Average		6.4	7.0	1.10	7.1	7.9	1.15

Statistical $P_{\Delta} > 0.4$

cubic centimeters of "A" plasma were placed in vessels numbered 2 and 4; 2.0 cc. of "T" plasma were placed in vessels numbered 5 and 6. Two slices of liver were placed in vessels numbered 4 and 6.

To the characteristic inset well of each vessel 0.2 cc. of 10 per cent sodium hydroxide was added. The vessels were filled with oxygen and placed in the water bath at 37.5° C. at 4:54 P.M. and then shaken 50 times a minute. Readings were begun at 5:10 P.M. In each vessel the pressure change, h, was multiplied by the vessel constant, k, to obtain the figure for the oxygen consumed, x. The blanks on "A" and "T" plasma were subtracted from the x value of vessels 4 and 6, respectively. At the conclusion of the experiment the liver slices were removed, dried at 100° C. for two hours, and weighed. The data are shown in Table 7.

TABLE 7. PROTOCOL II. EXPERIMENT 178. OXYGEN CONSUMPTION OF MOUSE LIVER SLICES DETERMINED IN THE PLASMA OF A CONTROL (ALKALI-INJECTED) AND A THYROXINE-INJECTED GUINEA PIG, RESPECTIVELY. VESSELS 2 AND 4 CONTAINED CONTROL PLASMA, 5 AND 6 PLASMA FROM THYROXINIZED ANIMAL. VESSELS 4 AND 6 CONTAINED MOUSE LIVER.

Vessels filled with oxygen; 0.2 cc. 10% NaOH in inset; temperature 37.5°C.; mouse liver in 2 cc. of guinea pig plasma.

Vessel	2		4				5		6			
Vessel volume	11.48		11.49				12.96		13.42			
Vessel constant	0.82		0.82				0.95		0.99			
Tissue weight			8.1						6.7			
Time	*h*	*x*	*h*	*x*	*x'*	*x'*/mg.	*h*	*x*	*h*	*x*	*x'*	*x'*/mg.
10	9	7	39	32	25	3.1	9	9	33	33	24	3.6
20	13	11	72	59	48	5.9	14	13	63	62	49	7.3
30	15	12	101	83	71	9.8	17	16	90	89	73	10.9
60	21	17	185	152	135	16.7	24	23	173	171	148	22.1
80	24	20	241	197	177	21.9	26	25	223	221	196	29.3
100	25	21	295	242	221	27.3	28	27	275	272	245	36.6
120	27	22	349	286	264	32.6	30	29	324	321	292	43.6

The corrected values for x were divided by the weight of the dried tissue and the Qo_2, or cubic millimeters of oxygen per hour per milligram of dried tissue was determined. The Qo_2 in "A" plasma was 16.0; in "T" plasma, 22.0. The elevation in the hyperthyroid plasma was, accordingly $(22.0-16.0)/16.0=37\%$.

Experimental Data. In Table 8 are given the measurements of oxygen consumption for slices of liver rapidly removed from normal and thyroxine-treated animals, respectively. These data merely demonstrate the well-established finding (Meyer, McTiernan and Aub, 1933) that the surviving tissues of hyperthyroid organisms exhibit an abnormally high oxygen consumption after excision. Comparison of the means of each group showed the difference to be significant ($P<0.02$).

TABLE 8. OXYGEN CONSUMPTION IN RINGER-PHOSPHATE-GLUCOSE OF LIVER SLICES FROM CONTROL (ALKALI-INJECTED) AND THYROXINE-TREATED GUINEA PIGS RESPECTIVELY

Exp.	Qo_2		Difference	Per Cent
	Control	Hyperthyroid		
302	2.8	4.9	2.1	74
301	4.3	6.7	2.4	56
300	4.9	3.6	−1.3	−26
299	3.3	4.3	1.0	30
298	3.0	4.2	1.2	40
297 (1)	2.4	4.4	2.0	83
(2)	3.7	3.9	0.2	5
296	4.3	4.9	0.6	14
292	4.0	4.9	0.9	22
244	3.3	4.2	0.9	27
Average	3.6	4.6	1.0	28

Statistical $P<0.02$.

TABLE 9. OXYGEN CONSUMPTION OF MOUSE LIVER IN THE PLASMA OF CONTROL (ALKALI-INJECTED) AND THYROXINIZED GUINEA PIGS. ON THE LEFT SIDE OF THE TABLE ARE GIVEN THE TOTAL DOSAGE AND THE NUMBER OF DAYS OF ADMINISTRATION OF THYROXINE TO THE GUINEA PIGS WHOSE PLASMA WAS USED AS THE MEDIUM

No.	Exp.	Thyroxine		Qo_2		Increase	Percentage increase
		Total dose mg.	Days injected	A	T		
1	178	1.54	4	16.7	22.1	5.4	32
2	179	1.92	5	14.0	16.5	2.5	18
3	196	1.54	4	14.4	17.9	3.5	24
4	199	3.04	9	12.9	13.6	0.7	5
5	207	1.92	5	16.4	17.7	1.3	8
6		1.54	4		22.7	6.3	39
7		1.15	3		22.2	5.8	35
8		0.77	2		19.5	3.1	19
9		0.39	1		20.4	4.0	24
10	212	1.92	5	17.0	19.0	2.0	12
11		1.54	4		18.2	1.2	7
12		1.15	3		22.8	5.8	34
13	222	1.54	5	24.2	16.7	−7.5	−31
14	223	0.77	2	20.2	24.0	3.8	19
15	226	1.15	3	15.0	17.5	2.5	17
16	236	0.39	1	15.5	19.6	4.1	26
17	237	0.39	1	14.7	17.6	2.9	20
18	238	0.77	2	15.9	20.4	4.5	28
19	292	1.54	4	19.2	17.9	−1.3	− 7
20	293	0.39	1	11.7	16.2	4.5	38
21		0.19	1		19.2	7.5	64
22	294	0.19	1	14.4	18.1	3.7	26
23		0.39	1	15.6	18.2	2.6	17
24	295	0.19	1	20.6	19.2	−1.4	− 7
25		0.38	1	17.1	18.3	1.2	7
26	296	0.19	1	14.4	15.4	1.0	7
27	297	0.58	2	13.5	17.4	3.9	29
28	298	0.77	2	15.5	17.5	2.0	15
29	299	0.77	2	15.0	18.5	3.5	23
30	300	0.77	2	15.2	18.0	2.8	18
31	301	0.77	2	16.1	16.4	0.3	2
Number of observations				24	31		
Average				16.1	18.7	2.6	18
Standard deviation				±2.7	±2.3	±2.5	

P ("T" vs. "A") <0.001. $P(\Delta Qo_2) < 0.05$

In Table 9 are given data for normal livers (a) with and (b) without treatment with the blood of hyperthyroid animals. These may be contrasted with data in Table 8. Whereas liver from a hyperthyroid animal shows an elevation of 28 per cent above the control, the vicarious response shows an elevation of only 18 per cent (Table 9). Statistical analysis of the respective values for the control blood and the hyperthyroid blood show the difference to be highly significant ($P < 0.001$). When the differences (ΔQo_2) for each individual experiment were analyzed, they were likewise found to be significant ($P < 0.05$).

The findings indicate that, like tumor tissue, normal surviving

liver tissue likewise gives a vicarious metabolic response when incubated with the blood of a hyperthyroid animal. It is interesting that no considerable incubation in the hyperthyroid blood is required in the case of liver tissue. These determinations in plasma were made by the single vessel method, which involves loss of carbon dioxide from the plasma. This loss results in a shift in pH to the alkaline side, i.e., to pH 8.5 or even to 9.0. This degree of alkalinity is obviously unphysiological for the entire organism, but it does not affect unfavorably the oxygen consumption of isolated liver tissue, according to Canzanelli, Greenblatt, Rogers and Rapport (1939).

Pure thyroxine added to plasma.—With both liver and sarcoma 180, no response could be demonstrated when pure thyroxine was added directly to normal plasma. The drug was added in the form of a concentrated solution, so as to avoid alteration of the medium through dilution. Various periods of incubation were tried without effect. Unfortunately after some 6 hours of incubation at 37° C., or some 10 hours (for liver) in the ice box, a sharp rise in oxygen consumption occurs in control surviving tissue. For example, in experiment 24 values for h for liver slices in RPG were 12, 4, 68 and 29 mm. per hour after 1, 6, 11, 23 and 25 hours respectively at 37.5° C. Through the use of arachnid heart, Davis and Hastings (1936) were able to avoid this complication.

DISCUSSION

In three series of experiments with sarcoma 180 the average increase in metabolism following treatment with hyperthyroid blood, as compared with control blood, was 20 per cent. In spite of the high degree of variability in the data, the increments were on the plus side to a preponderant degree and the probability that the difference between the "A" series and the "T" series was due to chance was less than 1 in 100 in most cases. It is evident, then, that the hyperthyroid blood during the incubation period was able to alter the tissue in such a way as to produce a calorigenic effect that persisted during the final determination.

Identification of the active substance in the blood has not been made. Current views of the nature of the thyroid hormone make it unlikely that either thyroxine or thyroglobulin *per se* is involved. The most reliable experiments with thyroxine *in vitro* involve a latent period of 12 hours before any increase in metabolism is observed (Davis and Hastings, 1936). Several laboratories have confirmed this result, although most of the investigators have used Ringer's medium (or variants thereof) rather than plasma. Moreover, as Davis and Hastings (1936) pointed out, the lag indicated the use of organs from cold-blooded animals. In the experiments reported here, control observations were made with normal mammalian plasma reinforced with small additions of a relatively concentrated solution of thyroxine.

The results were frankly negative, as was expected; but they minimize the possibility of an artificial factor in the present findings.

Canzanelli and Rapport (1937) have investigated the immediate *in vitro* activity of thyroglobulin, thyroxine and related compounds. Thyroglobulin was the only one observed to influence tissue metabolism. Thyroglobulin, however, does not appear in systemic blood in quantities sufficient to be detected by immunological methods (Lerman, 1940).

An immediate effect was obtained with mouse liver tissue by making the determination in a medium of plasma obtained from control and thyroxine-injected guinea pigs, and eliminating the incubation period. Although this simplified the experimental situation, there was no improvement in reliability of any single determination and the pH was left uncontrolled at a slightly alkaline level.

It might be suspected that the difference between "A" and "T" quotients was due simply to differential rates of death in the two sets of surviving tissue slices. Any surviving tissue is constantly undergoing "agonal" decline in metabolism, following which there occurs a violent uncontrolled oxidation of many tissue substrates. Could it be possible that hyperthyroid plasma contains a non-specific substance which delays for a time the inevitable decline toward death? This possibility seems unlikely in view of the data summarized in Table 5. In those experiments it was shown that the vicarious metabolic response induced by the "T" blood exceeded the initial level of oxygen consumption in the untreated tissue, as well as in the tissue treated with normal ("A") blood. This finding indicates a bona fide vicarious response—not merely a failure to decline.

An alternative explanation of the findings reported herewith might be that hyperthyroidism induces a change in the *milieu intérieur* which in itself can influence cellular oxidations. Such a change, if significant, would be independent of a direct effect of an iodine compound on cell metabolism. Several chemical alterations of plasma in extreme hyperthyroidism, indeed, have been described (Maddock *et al.*, 1937). On the whole, however, such findings do not afford a ready explanation of the increased rate of oxygen consumption.

SUMMARY AND CONCLUSIONS

It has long been known that many excised tissues from animals treated with thyroxine show a high metabolic rate. It now appears that the blood of *hyperthyroid* animals can induce an elevated metabolic rate in surviving tissues excised from normal animals. This vicarious metabolic response to hyperthyroid blood has been demonstrated with the blood of mice and guinea pigs for both liver tissue and sarcoma 180. Pure thyroxine added to normal mammalian blood did not induce this metabolic response under the experimental conditions. The findings do not, of themselves, indicate the nature of the

circulating thyroid hormone. Combined, however, with collateral data on the iodine-containing fractions in hyperthyroid plasma, they provide evidence for the view that the circulating thyroid hormone is neither thyroxine nor thyroglobulin in pure form.

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MITOSIS STIMULATION IN THE THYROID GLAND INDUCED BY THIOURACIL

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ADMINISTRATION of thiouracil to normal rats produces a two-fold effect on the thyroid gland: (1) inhibition of the formation of thyroid hormone and (2) stimulation of growth of the gland. The latter occurs in response to excessive secretion of thyrotrophic hormone resulting from decreased secretion of thyroid hormone (Mackenzie and Mackenzie, 1943; Astwood *et al.*, 1943). The morphological changes in the thyroid consist in enlargement (hypertrophy) and multiplication (hyperplasia) of the acinar cells, and are characterized by increased size and weight of the gland, increased height and papillary folding of the acinar epithelium and loss of follicular colloid.

The experiments reported here represent an attempt to investigate the time of occurrence and extent of the hyperplastic component of this phenomenon. The colchicine technic was employed for this purpose.

METHODS

Seventy adult rats were employed in this study; all received a stock diet of purina dog chow with lettuce added twice weekly. In the great majority of instances, thiouracil was given in 0.05% concentration in the drinking water. In some cases, 10 cc. of the thiouracil solution was given by stomach tube twice daily.

The animals were kept at 21–24° C. with the exception of those used to study the influence of extremes of temperature. The latter were kept at 37° and 10° C., in an incubator room and cold room, respectively. Eight hours before being sacrificed, each animal received colchicine subcutaneously (0.1 mg. per 100 gm. body weight).

The thyroid glands were dissected free of connective tissue and were rapidly weighed on a torsion balance. They were fixed in Bouin's fluid and paraffin sections were stained with hematoxylin-eosin. A piece of intestine was fixed and sectioned in the same manner in each case, serving as a control for the colchicine effect.

The animals were divided into four groups: (1) 38 rats, kept at 21–24° C., received thiouracil for 1–44 days; (2) 2 rats, kept at 21–24° C., received thiouracil for 6½ and 7½ months, respectively; (3) 9 rats were kept at 37° C. for 5 days, 5 receiving thiouracil and 4 serving as control; (4) 21 rats were kept at 10° C. for 5 days, 12 receiving thiouracil and 9 serving as control.

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[1] J. Ewing Mears, Fellow in Physiology and Medicine.

RESULTS

In the group maintained at ordinary temperatures (21–24° C.) mitosis stimulation was pronounced at 5 and maximal at 10 days, diminishing strikingly after 15 days. Evidence of stimulation was observed in 4 of 15 rats receiving thiouracil for 1–3 days, the earliest occurring at 24 hours. Some of the thyroid glands in this group (1–3 days) exhibited slight thinning of colloid and increase in cell height, neither of these phenomena, however, being related consistently to the occurrence of mitosis stimulation.

Five animals sacrificed at 5, 10, 21, 37, and 44 days respectively, showed no evidence of significant mitosis stimulation. The glands of all treated animals were heavier than those of the controls. Only an occasional mitotic figure was observed in the two rats treated for $6\frac{1}{2}$ and $7\frac{1}{2}$ months, respectively, the usual histologic evidence of stimulation (increase in cell height and loss of colloid) being also less pronounced than in animals treated for relatively short periods.

Only 1 of the 9 rats kept at 37° C. showed evidence of mitosis stimulation and this was of minimal degree. The thyroids of the controls showed some flattening of the epithelium and increase in colloid. Marked stimulation was evident in all of the treated animals kept at 10° C., the controls showing none. The thyroids of the latter controls differed in no significant respect from control animals kept at 21–24° C. with regard to cell-height and follicular colloid.

COMMENT

These observations indicate that hyperplasia (cell multiplication) occurs in the thyroid early in the course of thiouracil administration (24 hours), increases to a maximum at 10–15 days and then decreases. The secondary diminution in mitotic activity would be anticipated inasmuch as new formation of cells during this period serves only to maintain the number of cells present at the height of the hyperplastic reaction. However, this observation is of interest in connection with the question of the possibility of inducing malignancy in goiters exposed to thiouracil for long periods of time.

There is ample evidence that exposure to low environmental temperatures increases and to hot temperatures decreases thyroid functional activity. It has been shown (Dempsey and Astwood, 1943) that the response of the thyroid to thiouracil is accelerated in a cold (4° C.) environment and is retarded at incubator temperatures. This is explained on the basis of more rapid utilization of preformed thyroid hormone at the lower temperature. These findings are corroborated by our observations, mitosis stimulation being pronounced relatively early in the chilled animals (10° C.) and practically absent at 37° C. It should be pointed out that the "cold experiments" of Dempsey and Astwood were carried out at 4° C. and ours at 10° C.

The fact that the thyroids of the control rats at the latter temperature did not differ materially from those kept at ordinary temperatures, increases the significance of the marked stimulation induced by thiouracil under these conditions.

As might be anticipated in some instances evidence of "hypertrophy" (increased cell height) and loss of colloid were present before mitotic activity was demonstrable. However, the latter appears early in the course of thiouracil administration, presumably in response to thyrotrophic stimulation. In this respect, the response of the thyroid resembles that of the epithelium of the female genital tract to estrogens (Allen, 1937) and of the prostate to androgens (Fleischman, 1939). In each case, the stimulating hormones induce "growth" of the target organs as an early effect.

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ADRENAL MEDULLA IN WATER DIURESIS AND WATER INTOXICATION[1,2]

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THE FACT that adrenalectomy reduces markedly the diuretic response to water and increases the susceptibility to water intoxication has generally been attributed exclusively to adrenal cortical and not medullary deficiency. Eversole, Edelmann and Gaunt (1940) noted some deficiency in handling water in rats with one adrenal removed and the second demedullated, but since this was easily repaired by cortical extract, it was taken as evidence of a slight functional incapacity of this single regenerated cortical gland. Other evidence is available that one adrenal cannot maintain animals during stress as well as two (Horvath, Hitchcock and Hartman, 1938; Ingle, 1940).

It is known, however, that epinephrine injections affect renal circulation (Richards and Plant, 1922 a, b; Chasis, Ranges, Goldring and Smith, 1938). Both polyuria (Richards and Plant) and oliguria (Rydin and Verney, 1938; Chasis, *et al.*) have been reported to follow the use of epinephrine, and according to Toth (1938) which result is seen depends upon dosage and rapidity of administration. This author found polyuria an invariable consequence of subcutaneous or intramuscular injections in the dog, but this is apparently not necessarily true in man (Chasis *et al.*, 1938). Silvette (1942), using huge doses of epinephrine, increased the diuresis resulting from intraperitoneal saline administration in rats.

Stein and Wertheimer (1944) have recently reported experiments showing a diminished diuretic response to water and a "markedly enhanced susceptibility to water intoxication" in demedullated animals—a deficiency relieved by epinephrine injections. They found the medullary hormone to have, furthermore, similar diuretic and protective effects when given to adrenalectomized animals.

Their results, although interpreted with caution and restraint, obviously suggested the possibility, first that the adrenal medulla may

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[2] While this work was in progress, it was learned that Hays and Mathieson were doing similar experiments with epinephrine in adrenalectomized animals. Their results and ours were for the most part mutually confirmatory, and at our request have been printed together.

be a physiological factor of considerable importance in the regulation of water metabolism; and secondly, that the well-known deficiencies in adrenalectomized animals may be due in considerable part to loss of the medulla.

At the time their report appeared, we had observed, but not reported, effects of epinephrine similar in general to those of Stein and Wertheimer. Since then, these observations have been extended and a reinvestigation made of the effects of adrenal medullectomy in animals in which both adrenal cortices were left *in situ*. The results and their implications are reported here. A preliminary abstract has been published (Gaunt, Cordsen and Liling, 1945).

METHODS

The rats used approximated 175 grams in body weight and were mostly females. Their response to two or five doses of distilled water at hour intervals or to 13 doses given at half-hour intervals (if they survived long enough to receive the full dosage) were studied by methods previously published (Gaunt, 1944; Gaunt, Cordsen and Liling, 1944; Liling and Gaunt, 1945). The water dosage was calculated on the basis of body surface—3 cc. per 100 sq. cm., and for a 175 gram rat each dose was 8 cc. The calculation of dosage on the basis of body surface probably minimizes variability due to differences in body size, but nevertheless control and treated animals of closely matched weight were used.

Adrenal medullectomy was performed by the method of Evans (1936), i.e., the capsule of the gland was nicked and the pulp including all of the medulla and most of the cortical tissue was squeezed out. From the remaining capsule a gland of purely cortical tissue regenerates (literature cited by Eversole, *et al.*, 1940). A drinking solution of 1% NaCl was used for a week after operation. Tests were made between the 24th and 77th days after demedullation. Sham-operated controls were not run because their normality in response to water had previously been established (Gaunt, 1944). Tests on adrenalectomized rats were made either 18 hours (1 day in the tables and graphs) or 7 days after operation.

In studying the resistance to excess water emphasis was placed on changes in body temperature, the fall of which provides a consistent and reliable index of the extent and degree of a state of water intoxication. Records were also kept on the occurrence of convulsions and collapse, but these data, like those on survival, are less reliable quantitatively than those on temperature. Mortality figures are based on deaths occurring within 24 hours of the end of an experiment.

In all experiments detailed here, epinephrine[3] was given subcutaneously with mild massage, in suspension in oil. The volume of oil was 0.06 to 0.075 cc. per 100 grams body weight, and injections were made just before the first dose of water. The presence of continuing over-dosage symptoms indicated that epinephrine given in this way was effective for several hours. Aqueous epinephrine, used less estensively, did not give greatly different results. Data are available on doses of adrenalin other than those figured in

[3] Adrenalin-in-oil kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co.

normal and adrenalectomized animals, and with smaller doses of water in demedullated animals. Since they added nothing that was not otherwise apparent, details are omitted.

Appropriate controls, in approximately equal numbers, were always run simultaneously with the various experimental groups, and it was required that an effect to be considered significant must have been seen consistently

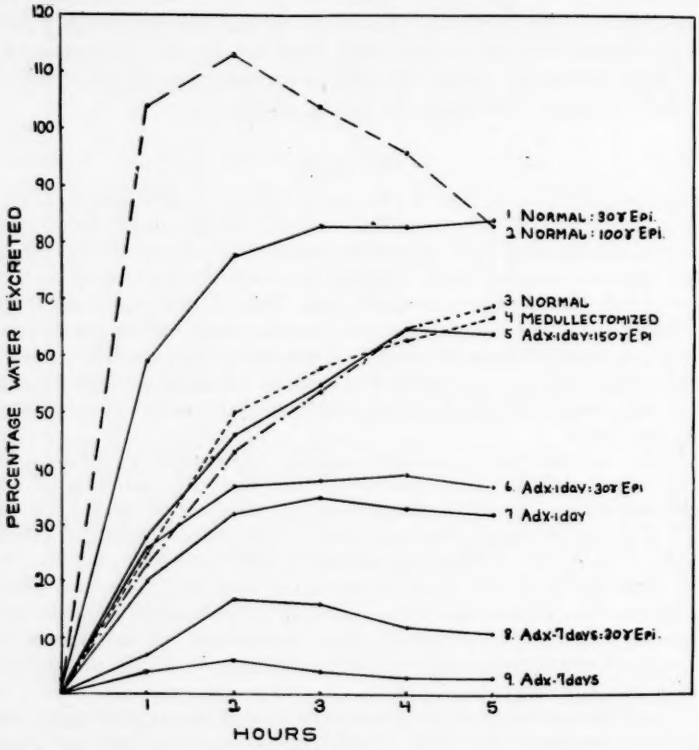

FIG. 1. Showing diuresis curves in rats during the administration of 5 doses of water approximating 8 cc. each. The first dose was given at 0 hour and the subsequent ones at hour intervals. Results are shown for normal animals with and without injections of epinephrine ("Epi"), for adrenal medullectomized animals, and for adrenalectomized ("Adx") animals receiving various treatments. All animals adrenalectomized for 7 days had been maintained on 1% NaCl. Additional data are given in Table 1.

on different days with reference to controls observed at the same time. This served as a check on the rather high spontaneous variability seen in experiments of this type—statistical measures of which are given by Liling and Gaunt (1945). In the present experiments the differences between various groups were either very great or obviously insignificant, and hence statistical considerations are not included.

RESULTS

Effects of adrenal medullectomy (Fig. 1, Curve 4; Fig. 2 and Table 1). When given either 5 or 13 doses of water no deficiency in excreting the water or consistent signs of unusual susceptibility to the toxic effects of water were seen in demedullated animals. The same was true when two doses of water were given but figures are not included here. For convenience, control figures for all experiments are grouped together in the graphs, but actually, in the majority of cases, the rate

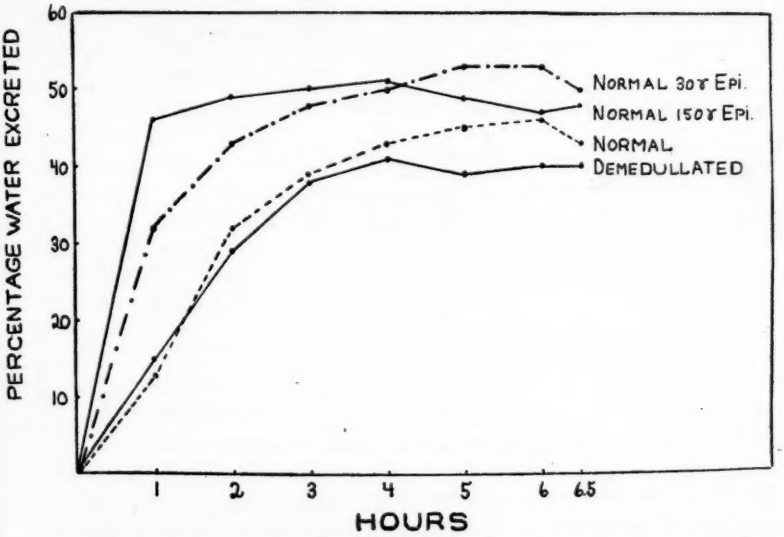

FIG. 2. Showing diuresis curves in rats during the administration of 13 doses of water approximating 8 cc. each. The first dose was given at 0 hour and subsequent ones at half hour intervals. Results are shown for normal animals receiving epinephrine, adrenal medullectomized animals, and normal untreated animals. The "normal" curve is made up of the controls of all experimental groups averaged together for simplicity. The apparent differences between the normal and demedullated is not significant and was not seen at all in the controls run simultaneously. Additional data are given in Table 1.

of diuresis in medullectomized rats receiving either small or large doses of water exceeded slightly that in their own controls run simultaneously. These differences were not significant but they serve to emphasize the absence of an adrenalectomy-like deficiency. The mortality in demedullated animals given 13 doses of water exceeded somewhat that of their normal controls. The number of deaths was well within normal limits nevertheless, and exceeded parallel controls because of an unusual mortality in one series only of operated animals.

Effects of epinephrine injections in intact animals (Fig. 1, Curves 1, 2; Fig. 2 and Table 1). A striking effect of epinephrine in increasing

diuresis in intact animals was seen when either two or five doses of water were used. With 100 micrograms of epinephrine per 100 grams body weight, the animals presented the anomalous situation of dehydrating themselves in the early stages of the experiments while water was being given in amounts which untreated animals could not excrete (Fig. 1, Curve 2).

When 13 doses of water were given, epinephrine had a lesser but

TABLE 1. EFFECT ON RESPONSE TO WATER INGESTION OF ADRENAL MEDULLECTOMY AND OF EPINEPHRINE INJECTIONS IN NORMAL AND ADRENALECTOMIZED RATS

Treatment	No. Rats	Water Excretion Shown in:	Body Temperature (F.°) at Following Hours			% Died	Cl. Excretion (as Mg. NaCl)
			3rd	5th[3]	6.5[3]		
Series 1. Rats Receiving 5 Doses of Water: Effect of Medullectomy and Epinephrine							
Normal: Untreated	34	Fig. 1, Curve 3	98.6	98.1		0	14.7
Adrenal Medullectomy	25	Fig. 1, Curve 4	98.5	98.1		0	15.1
Normal: 30γ Epi./100 gm.	16	Fig. 1, Curve 1	97.6	97.9		0	31.2
Normal: 30γ Epi.+.1u Pitressin /100 gm.	8	Fig. 3, Curve 3	96.5	91.0		0	70.5
Normal: .1u Pitressin/100 gm.	8	Fig. 3, Curve 6	96.1	89.5		0	44.6
Normal: 100γ Epi./100 gm.	14	Fig. 1, Curve 2	97.1	98.1		0	44.6
Series 2. Rats Receiving 13 Doses of Water: Effect of Medullectomy							
Adrenal Medullectomy	15	Fig. 2	94.3	89.5(8)	88.6(6)	80	
Normal: Untreated	14	Fig. 2	94.8	90.8(8)	88.1(7)	57	
Series 3. Rats Receiving 13 Doses of Water: Effect of Epinephrine							
Normal: 30γ Epi./100 gm.	14	Fig. 2	94.4	92.6(5)	89.0(10)	50	69.2
Normal: 150γ Epi./100 gm.	12	Fig. 2	94.5	91.4(9)		75	63.7
Normal: Untreated	21	Fig. 2	95.1	92.4(15)	88.6(6)	48	55.3
Series 4. Adrenalectomized Rats Receiving 5 Doses of Water: Effect of Epinephrine							
Adx.-1 Day: 150γ Epi./100 gm.	6	Fig. 1, Curve 5	97.0	95.1		0	
Adx.-1 Day: 30γ Epi./100 gm.	6	Fig. 1, Curve 6	97.5	93.1		0	
Adx.-1 Day	11	Fig. 1, Curve 7	97.4	93.5		0	
Adx.-7 Days[1]: 30γ Epi/100 gm.	8	Fig. 1, Curve 8	95.1	87.1		100	
Adx.-7 Days[1]	8	Fig. 1, Curve 9	94.3	87.3		87	
Adx.-7 Days[2]	4	Fig. 3, Curve 7	95.7	90.3		0	
Adx.-7 Days[2]: 5 mg. DCA	5	Fig. 3, Curve 5	96.5	92.5		0	
Adx.-7 days[2]: 30γ Epi./100.+5 mg. DCA	5	Fig. 3, Curve 4	96.3	93.5		0	

[1] Salt-maintained.
[2] DCA-maintained.
[3] Figures in parenthesis indicate the number of animals on which determinations were made if less than total number of group.

distinct effect on diuresis in the early stages, but this faded off to insignificance toward the end of the experiment. Despite the initial advantage given by this augmented diuresis, epinephrine treatment—unlike that previously seen with cortical hormones—did not afford any protection against the intoxicating effects of these large doses of water as judged by the maintenance of body temperature, by survival or by the prevalence of collapse and convulsions. The body temperatures in this group, treated as well as controls (Series 3 of Table 1), were reduced less than we customarily observe in experiments of this type. The reason is not known, but the point of importance is the exact parallelism between normal and treated animals run simultaneously.

All of the effective doses of epinephrine used produced obvious signs of overdosage: stretching, gasping, etc. The only dose of the medullary hormone which did not produce such symptoms was 3 micrograms per 100 grams body weight, and that was without effect on diuresis. The effects noted above were, therefore, probably limited to nonphysiological amounts of this hormone.[4]

In contrast to these results, Stein and Wertheimer saw only very slight effects of epinephrine in intact naimals.

Effect of epinephrine in adrenalectomized rats (Fig. 1, Curves 5, 6, 8; Table 1). Epinephrine injections increased markedly the diuretic response to water in adrenalectomized rats, but not to the extent that it did in normal rats—a finding in agreement with Stein and Wertheimer, and Hays and Mathieson (1945). A maximum of five doses of water was used, since this is adequate to produce intoxication after adrenalectomy.

In animals operated only one day (Fig. 1, Curve 5), a large dosè of epinephrine (150 micrograms per 100 grams) restored diuresis to normal ranges—an effect which resembled that of cortical hormones, and is to be contrasted with the ineffectiveness of other diuretic influences (i.e. thyroxin, Gaunt, *et al.* 1944; and water adaptation, Liling and Gaunt 1945) in adrenalectomized rats.

In animals operated for 7 days, the same effect was present (Fig. 1, Curve 8). A more striking result than those figured here was seen in animals adrenalectomized for seven days and given two doses of water and 150 micrograms of adrenalin per 100 grams body weight. At the third hour they had excreted 48 per cent of the water given (normal animals would have excreted about 80 per cent) whereas their salt-treated adrenalectomized controls had excreted only 4 per cent.

It is to be remembered that animals adrenalectomized for a week, even though maintained during that time on salt, desoxycorticosterone or cortical extract, are almost incapable of excreting any water administered by stomach tube; they are, furthermore, relatively refractory to large replacement doses of desoxycorticosterone and somewhat so to cortical extract (Gaunt, 1944; Hays and Mathieson, 1945; see also Fig. 1, Curve 9 and Fig. 3, Curve 7). It is, therefore, of interest that adrenalin could exert any effect at all in these cases.

In our results there was one notable difference between the effects of adrenalin and those previously found for cortical hormones on adrenalectomized animals given water: while either would increase the sluggish diuresis, only cortical hormones helped consistently in preventing the characteristic temperature-reducing effects of excess

[4] All results reported in detail here concern the effects of epinephrine in animals given water. In addition it was found in 16 cases that both 30 and 150 micrograms of epinephrine per 100 grams body weight clearly increased the rate of water excretion in normal fasted rats that were allowed water *ad lib.* during the fast but given no water by stomach tube. The conditions under which this effect was observed did not, however, permit accurate quantitative determinations of the small amounts of urine involved.

water. Likewise in our 7-day operated animals epinephrine did not increase survival. It is apparent, however, from the figures of Hays and Mathieson that if the water dosage and other circumstances are such as to induce a high mortality in adrenalectomized rats a life-maintaining effect of adrenalin after excess water can be demonstrated.

Effects of adrenalin plus desoxycorticosterone acetate in adrenalectomized rats. Since diuresis can be enhanced in 7-day adrenalectomized animals by either adrenalin or desoxycorticosterone (cortical extract is more effective—Gaunt 1944) the effects of the two combined were studied. Only one series of animals was run because we found that this experiment had already been done by Hays and Mathieson and our first results confirmed theirs.

Fifteen rats were adrenalectomized and maintained for a week on doses of desoxycorticosterone acetate[5] varying from 0.5 to 1.0 mg. per day, the dose being adjusted to maintain fully normal weight increments. The animals were then divided into three groups for tests with 5 doses of water.

Group 1 (Fig. 3, Curve 7; Table 1) received only the regular maintenance dose of desoxycorticosterone acetate preparatory to the water tests.

Group 2 (Fig. 3, Curve 5; Table 1) received 5 additional milligrams of desoxycorticosterone acetate, half of which was given 18 hours, and the other half 30 minutes before the water.

Group 3 (Fig. 3, Curve 4; Table 1) received desoxycorticosterone acetate as in group 2 and also 30 micrograms of adrenalin per 100 grams body weight.

It was clear that the two hormones together provided a more effective replacement as far as diuresis was concerned than either alone.

Epinephrine might be expected to be more effective in the presence of cortical hormones because the latter provide effective resistance against water intoxication and maintain the animals in good condition.

Effects of epinephrine on vasopressin-inhibited diuresis (Fig. 3, Curves 1, 3, 6; and Table 1). Normal rats given only 5 doses of water and 0.1 unit of pitressin tannate-in-oil per 100 grams body weight show a greatly inhibited diuresis and full symptoms of water intoxication (Liling and Gaunt, 1945).

Experiments were conducted to see if adrenalin could antagonize these effects of pitressin.[6] The experimental result was clear: epinephrine overcame to a marked extent the antidiuretic effects of

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[5] Desoxycorticosterone acetate (Percorten) kindly supplied by Dr. H. W. Hays of the Ciba Pharmaceutical Products, Inc.

[6] Pitressin tannate-in-oil kindly supplied by Dr. Oliver Kamm of Parke, Davis and Co.

pitressin, but again helped little if any in preventing the temperature-reducing effects of excess water. This is in contrast to other influences, e.g., the adaptation to water administration, which can ameliorate water intoxication symptoms without increasing diuresis after treatment with pitressin (Liling and Gaunt, 1945).

Chloride excretion (Table 1). Under conditions seen here, epinephrine seemed to increase chloride excretion both in terms of concen-

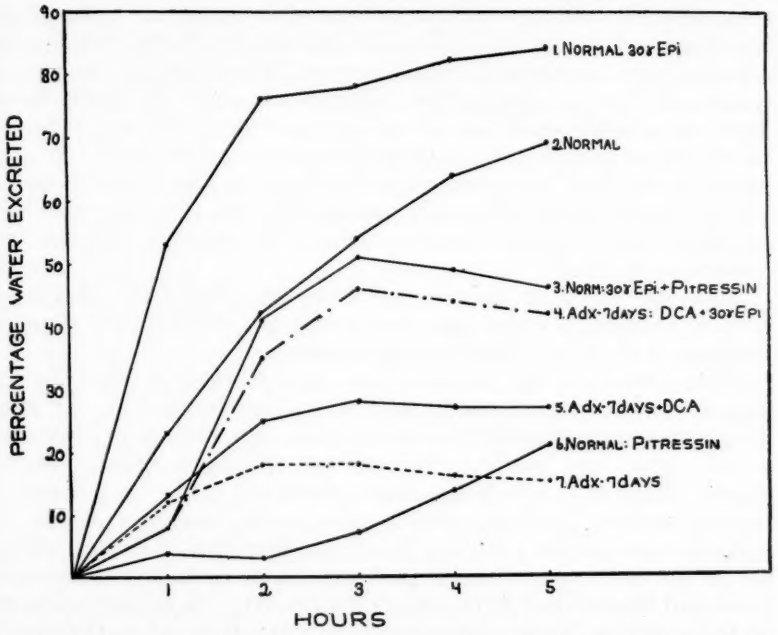

FIG. 3. Showing diuresis curves in rats during the administration of 5 doses of water approximating 8 cc. each. The first dose was given at 0 hour and subsequent ones at hour intervals. Results are shown for normal untreated animals (2) together with those receiving pitressin alone (6), epinephrine alone (1), and the two combined (3). Also shown, are curves for adrenalectomized animals maintained on desoxycorticosterone (7), animals similarly treated but receiving extra desoxycorticosterone before the water tests (5), and those receiving both desoxycorticosterone and epinephrine (4). Additional data are given in Table 1.

tration and total amounts in all groups of intact animals. This is in agreement with Stein and Wertheimer, but possibly not expected from older observations under different circumstances (Toth, 1937). The total chloride excretion in experiments where 13 doses of water were given is not of clear significance as far as renal function is concerned because the urine samples were often contaminated with diarrheal fluid; that was not the case with the smaller water doses. Adrenal medullectomized animals excreted chloride in normal amounts.

Pitressin is known to increase chloride excretion, and when given in combination with epinephrine, the effects of the two hormones were supplementary.

DISCUSSION

The pharmacologic effects of injected epinephrine in normal animals were clear in these experiments. It increased markedly the polyuria induced by water ingestion except when the water load approached toxic levels. Under proper circumstances, this diuretic effect was sufficient to cause dehydration even while water was being given in relatively large amounts. The increased water elimination in epinephrine-treated animals helped, however, little if any, in preventing symptoms of water intoxication. This may have been the result of the increased chloride excretion induced by treatment with epinephrine, which was presumably accompanied by similar losses of sodium, and would be expected to increase susceptibility to the toxic effects of water (Gaunt, 1944). Thyroxin, incidentally, has the opposite effect on chloride excretion and has a protective effect on water intoxication (Gaunt, *et al.*, 1944).

Epinephrine, in addition, is one of the few substances, other than cortical hormones, which can clearly increase the retarded diuretic response to water in adrenalectomized animals.

The question of first interest, however, is whether or not a physiological mechanism, normally used in water elimination, is being demonstrated by the effects of epinephrine injections in experiments of this type; and particularly, whether the deficiencies in water diuresis which have now been rather extensively studied in adrenalectomized animals, and ones which serve as the basis of a diagnostic procedure in Addison's disease (Robinson, Powers and Kepler, 1941), are in fact due in considerable part to adrenal medullary deficiency. Stein and Wertheimer have provided affirmative views and evidence on this question. Their conclusions were logically based on their findings of deficiencies in demedullated animals.

We have found no deficiency, on the contrary, in either diuresis or resistance to excess water in demedullated animals. If our finding is valid, then a heavy burden of proof rests on the proponents of the view that the effects of obvious overdoses of injected epinephrine depict a physiological phenomenon. We have previously presented evidence that the adrenal medulla is activated in water intoxication and that it is responsible for the blood sugar rises characteristically seen in that state (Gaunt, 1944). In none of our animals did it appear, however, that a normal water excretion depended on such an increased medullary activity.

No reason is apparent to explain the discrepancy between our results on medullectomized animals and those of Stein and Wertheimer. We have, however, called attention to a day-to-day variation in the

results of diuresis experiments which can be controlled only by comparing treated animals with appropriate controls run simultaneously. It is also essential, particularly if dosages of water are based on body weight, that control and experimental rats be of matched size. These are pointed out as potential sources of error; we have no reason to believe they were observed less carefully by Stein and Wertheimer than by us.

Were it not for the results in medullectomized animals a good argument could be made in support of the thesis that the deficiencies in adrenalectomized animals were the combined effects of loss of the cortex and medulla. This specificity of the diuretic action of epinephrine, an effect not shared by compounds of otherwise related pharmacological properties, has been noticed by Stein and Wertheimer and Hays and Mathieson. Previously in experiments on renal circulation Smith (1943) reported effects of epinephrine not given by ephedrine. This specificity would be consistent with a physiological role in such functions. The fact that epinephrine will supplement the action of cortical hormones in animals that have been adrenalectomized for several days would again be consistent with the idea that the two hormones normally work together in maintaining diuresis.

Whether the diuretic stimulus provided by mild doses of water is associated with, or due to, an increased epinephrine secretion is, however, another matter not at all demonstrated. If such does occur, then one has a physiological counterpart to the antidiuretic role of vasopressin in dehydration.

The action of cortical hormones in water diuresis is established both by deficiency and overdosage phenomena, and the only theoretical barrier to ascribing to them all adrenal action in such phenomena is the difficulty of replacement therapy in animals that have been adrenalectomized for several days.

Since no direct evidence is available, any comment on the mechanism of the diuretic action of epinephrine is purely hypothetical. This hormone has been thought to cause constriction of efferent renal arterioles and hence polyuria (Richards and Plant), and in man to reduce renal plasma flow, to increase the filtration fraction but not the filtration rate (Chasis, *et al.*). The dose used to effect these changes in the human kidney was close to that (3 micrograms per 100 grams) which was without effect on diuresis in the rat. The effect of variation in the dosage or method of administration on specific renal functions has not been studied, but they are such as to result either in oliguria or polyuria (Toth). The possibility that epinephrine affects tubular reabsorption of water has not been eliminated. It would not be expected that epinephrine as used here would increase blood pressure (Cori and Welch, 1942), and we confirmed this expectation in a few cases by direct measurement under nembutal anesthesia.

SUMMARY

The effects of adrenal medullectomy and of epinephrine injections on the diuretic response to water and the resistance to excess water given by stomach tube were studied in rats.

The response of medullectomized animals was similar to that of normal ones after the ingestion of either small or large amounts of water.

Epinephrine injections (30 to 150 micrograms per 100 grams body weight) greatly augmented the polyuria induced by hourly ingestion of the standard water dose in normal animals, and to such an extent in some instances that dehydration resulted while water was being administered. When water was given at half-hour intervals, the effect on diuresis was less pronounced and water intoxication was not prevented. Chloride excretion was enhanced in all instances by epinephrine.

Epinephrine increased diuresis in adrenalectomized animals given water but its effects were less marked in preventing symptoms of water intoxication.

Epinephrine supplemented the action of desoxycorticosterone acetate on the diuresis of adrenalectomized rats, and partially counteracted the antidiuretic action of vasopressin in normal rats.

It is concluded that the effects of adrenalectomy on water excretion are not due in significant part to loss of the adrenal medulla, and that any important physiological rôle of epinephrine in water diuresis is not established by these experiments.

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STUDIES ON WATER INTOXICATION IN ADRENALECTOMIZED RATS AND THE INFLUENCE OF DESOXYCORTICOSTERONE ACETATE (DCA) AND EPINEPHRINE IN WATER DIURESIS

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INTRODUCTION

DESOXYCORTICOSTERONE ACETATE (D.C.A.) has been shown to be effective not only in maintaining the life of bilaterally adrenalectomized animals but as a protective agent when normal and adrenalectomized animals are subjected to excessive amounts of water by mouth. This phenomenon of water intoxication has been described by Rowntree (1926) and it is now generally agreed that the cause of convulsions and death resulting from large amounts of water is due to excessive hydration of tissue cells with marked depletion of plasma electrolytes. Failure of adrenalectomized animals to excrete water normally when large amounts of water are ingested has been associated with adrenal dysfunction (Rigler 1935; Swingle *et al.* 1937; Gaunt *et al.* 1937; Shipley 1945; Stein and Wertheimer 1944; Hays and Mathieson 1945) but the mechanism is still not clear. This delayed diuresis following the ingestion of water has been used as a diagnostic procedure for Addison's Disease by Robinson, Power and Kepler (1941) and as an indication of adrenal function for animals bearing adrenal transplants (Eversole, Edelman and Gaunt 1940). It has also been used as an assay procedure for cortical extracts (Pebranyi 1941) and for urinary cortical materials (Schiller and Dorfman 1943).

This investigation was begun with the hope of establishing a quantitative assay procedure for desoxycorticosterone acetate and to elucidate some of the factors we believed to be concerned with water diuresis and adrenal function.

METHOD

Female rats, Wistar strain, weighing approximately 200 gms. were used in these experiments. Adrenalectomy was performed under ether anesthesia and in experiments extending beyond one day the rats were maintained on either desoxycorticosterone acetate or saline.

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Distilled water was given by stomach tube according to the formula: Body surface×4%×4 hourly injections.[1] For a 200 gm. rat the total water given was 50 cc. Adrenalectomized rats were given water at either one, five or eight day intervals following the operation. The volume of urine excreted was determined at hourly intervals and is expressed as per cent excretion of the sum total of water given at each hourly interval.

D.C.A., in oil, was injected subcutaneously in a volume of 0.1 to 0.3 cc. and in all cases the doses were total doses per animal. For one-day-adrenalectomized rats, D.C.A. was given in divided doses, one-half immediately after the operation and the remainder, sixteen hours before the water. For five and eight-day-adrenalectomized rats, the dose of D.C.A. was 1.0 mg. per day for three or six days respectively, and 1.0, 3.0 or 10.0 mg. in divided doses on the fourth or seventh day, the last injection being given sixteen hours before water was administered. Adrenalectomized control rats were allowed free access to 0.9 per cent sodium chloride solution, hereafter referred to as saline.

In the series of experiments where epinephrine HCl in aqueous solution was used, it was injected subcutaneously one-half hour before the administration of water. The dosage used throughout was 0.15 mg. per 100 gm. body weight and elicited only mild signs of overdosage, as evidenced in preliminary experiments on both normal and adrenalectomized rats. The moderate irritability and stretching movements disappeared after two or three hours. No difference in severity of symptoms could be observed between the two groups.

In order that more animals could be used in any one experiment, two rats receiving similar treatment were placed in one metabolism cage and the per cent excretion was calculated as the average for the two. Food was withheld 18 hours prior to and during the experiment, and no water was allowed except that given by stomach tube.

RESULTS

(a) *Water Diuresis in Rats Adrenalectomized one-day* (Table 1, Figure 1). In this series of experiments, normal and adrenalectomized rats were used as controls and it was found that at the end of seven hours, normal rats excreted 82 per cent; while adrenalectomized controls excreted 21 per cent of the water administered, with zero per cent mortality for the former, and 72 per cent for the latter group. Since it has been shown that cortical extract and D.C.A. (Swingle, 1937, 1941; Eversole, Gaunt and Kendall, 1942; Hays and Mathieson 1945), are effective in preventing symptoms of water intoxication we attempted to determine whether a dose-response relationship existed with D.C.A. With doses ranging from 0.2 to 10.0 mg. the per cent excretion was directly proportional to the dose but never reached the same level as that of normal controls. However, there was definite protection against convulsions and death from water intoxication, as evidenced by the fact that the 72 per cent mortality observed in the untreated adrenalectomized controls decreased to zero per cent for

[1] Body Surface = $B.W.^{2/3} \times 9.1$. Benedict's formula.

adrenalectomized rats which had been given 2 mg. or more of D.C.A. Smaller doses of D.C.A., which increased the per cent excretion only slightly over adrenalectomized controls, remained effective in protecting the rats from death due to water intoxication. While animals given 0.2 mg. of D.C.A. showed no mortality and those given only slightly higher doses, 17 to 22 per cent mortality (Table 1), actually 33 per cent of the rats on 0.2 mg. died by the end of 24 hours, with no increase in mortality for the other groups.

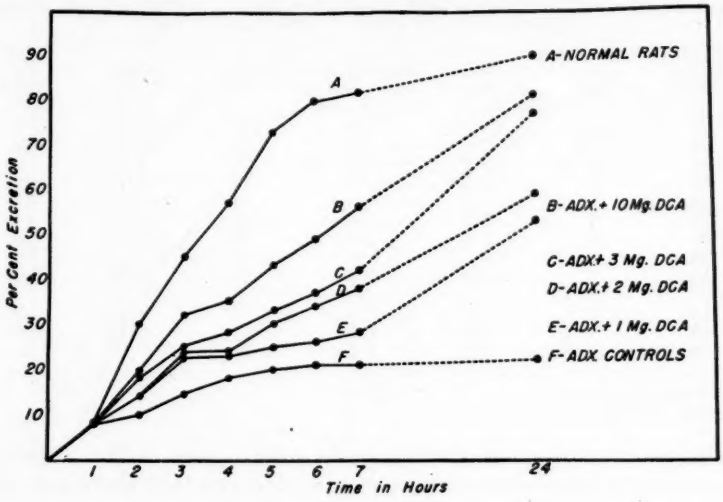

FIG. 1. Effect of varying doses of desoxycorticosterone acetate on water diuresis in rats adrenalectomized one day. Solid lines represent the per cent excretion from 0 to 7 hours; dotted lines 7 to 24 hours.

We have used this procedure as an assay for desoxycorticosterone acetate but the results of the experiments reported here show that despite the dose-response relationship, the individual responses remain extremely variable. It would be necessary to use large dose increments to increase significantly the diuretic response to water. Further studies are now in progress which may establish a quantitative method.

(b) *Water Diuresis in Rats Adrenalectomized Five and Eight Days and Maintained on D. C. A. or Saline.* It has been previously reported that while D.C.A. caused a marked diuresis in rats adrenalectomized for one day (Eversole *et al.* 1942; Hays and Mathieson 1945), its diuretic properties are lost in rats maintained for five or eight days (Gaunt 1944; Hays and Mathieson 1945). In our present experiments, (Table 2, Figure 3D and Figure 4E) the per cent excretion in rats maintained on 1 mg. per day of D.C.A. for four and seven days, was

no greater than that of the saline controls or for one-day-adrenalectomized controls. There was, however, a very marked difference between the two groups, for 62 per cent of the saline treated animals died as compared with only 8 per cent of the D.C.A. treated animals.

When additional hormone was given (Table 2; Figures 3C, 4F) on the day preceding the water, the response was again no greater than for those maintained on D.C.A. or saline, even with as much as 10 mg. of D.C.A.[2] This is in marked contrast to the increased diuresis in

TABLE 1. THE EFFECT OF DESOXYCORTICOSTERONE ACETATE AND EPINEPHRINE ON WATER DIURESIS IN RATS ADRENALECTOMIZED ONE DAY

No. of Rats	Days Adx.	DCA Mg.	Epinephrine Mg./100 Gms.	% Excr. 7 Hrs.	Mort. 7 Hrs.	% Mort.
27	—[1]	—	—	82	0/27	0
25	1[2]	—	—	21	18/25	72
18	1	0.2	—	26	0/18	0
18	1	0.5	—	32	4/18	22
18	1	1.0	—	28	3/18	17
18	1	1.5	—	42	3/18	17
20	1	2.0	—	38	0/20	0
36	1	3.0	—	42	0/36	0
16	1	10.0	—	56	0/16	0
10	1	—	1.5	64	0/10	0
10	1	1.0	1.5	68	0/10	0
9	1	3.0	1.5	79	0/9	0

[1] Normal controls
[2] Adx. controls

one-day-adrenalectomized rats treated with 10 mg. of D.C.A. where the excretion was 56 per cent. It will be observed that there was definite protection against death from water intoxication, even though the excretion was not increased. In experiments on absorption of pellets of D.C.A., to be published later, it was found that adrenalectomized rats maintained for forty days on pellets showed an even greater inability to excrete water. When additional hormone was given, this group excreted only nine per cent of the water even though they showed no signs of convulsions or death from water intoxication. At present no apparent reason is perceivable for this refractoriness in adrenalectomized animals on D.C.A. with normal growth increments.

(c) *Effect of D.C.A. and Epinephrine in one-day Adrenalectomized Rats.* Stein and Wertheimer (1944); Hays and Mathieson (1945) and Gaunt *et al.* (1945) have shown that epinephrine markedly increased the diuretic response to water in normal and adrenalectomized rats. Table 1, Figure 2, A, B, C, N, show the results of our experiments. Normal rats excreted 82 per cent of the water in seven hours, while adrenalectomized rats receiving 0.15 mg. epinephrine HCl per 100

[2] Gaunt (1944, 1945) reported some increase in diuresis in post adrenalectomized rats with desoxycorticosterone acetate. This discrepancy might be due to dosage as well as interval of injection.

gm. body weight excreted 64 per cent. When 1 mg. or 3 mg. of D.C.A. were given after adrenalectomy, and 0.15 mg. of epinephrine per 100 gm. body weight one-half hour before the water, the excretion increased to 68 and 79 per cent respectively. In Table 1 and Figure 1C it is shown that 3 mg. of D.C.A. alone caused an excretion of 42 per cent whereas the same dose of D.C.A. plus epinephrine increased the excretion to 79 per cent (Figure 2A). Stated in terms of per cent increase over controls, D.C.A. alone caused increased diuresis of 21 per

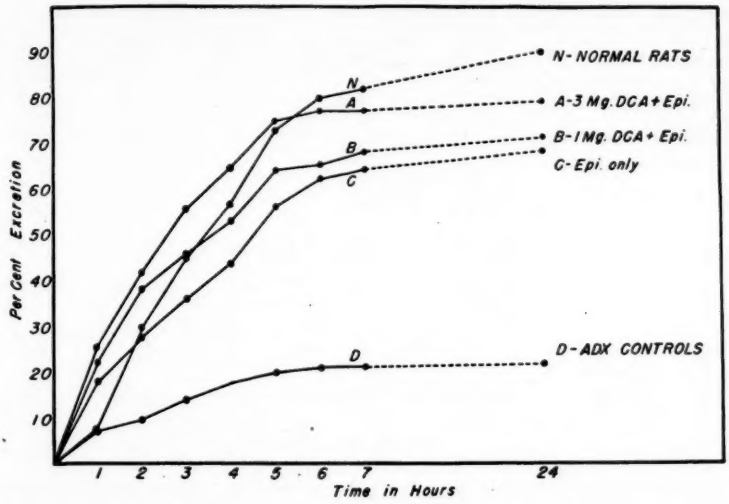

FIG. 2. Effect of desoxycorticosterone acetate plus epinephrine HCl on water diuresis in rats adrenalectomized one day. Solid lines represent the per cent excretion 0 to 7 hours; dotted lines, 7 to 24 hours. In curve C, epinephrine was given to untreated adrenalectomized controls.

cent, epinephrine alone, 43 per cent, and D.C.A. plus epinephrine, 58 per cent. It would seem evident that in this experiment there was an additive or synergistic effect of D.C.A. and epinephrine. In Figure 1 and 2 it will also be noted that epinephrine alone or in combination with D.C.A. resulted in a greater hourly rate of excretion than with D.C.A. alone, particularly in the first three hours.

(d) *Effect of D.C.A. and Epinephrine on Water Diuresis in Rats Adrenalectomized Five and Eight Days.* In section (b) it was shown that adrenalectomized rats maintained on D.C.A. or saline show the same inability to excrete water given by mouth as one day adrenalectomized controls. When epinephrine was given to rats maintained on saline for eight days, the excretion at the end of seven hours was 49 per cent (Table 2, Figure 4C); but when given to adrenalectomized rats maintained on D.C.A. (1.0 mg. per day) for five or eight days, the excretion increased to approximately that of normal controls

(Figure 3B, 4A). In comparing these results with those for one-day-adrenalectomized rats it will be noted that epinephrine was not as effective in increasing diuresis when rats were maintained on saline as it was in the case of the one-day-adrenalectomized controls. However, when epinephrine was given to five or eight-day-adrenalectomized animals maintained on a dose of D.C.A. which alone had no effect in increasing the diuresis, the excretion equaled that of normal

TABLE 2. THE EFFECT OF DESOXYCORTICOSTERONE ACETATE AND EPINEPHRINE ON WATER DIURESIS IN RATS ADRENALECTOMIZED FIVE AND EIGHT DAYS

No. of Rats	Days Adx.	DCA Days × Mgs.	Epinephrine mgs/100 gms.	% Excr. 7 Hrs.	Mort. 7 Hrs.	% Mort.
6	5	4×1.0	—	20	0/6	0
20	5	3×1.0 1×3.0	—	22	2/20	10
6	5	3×1.0 1×10.0	—	21	0/6	0
10	5	4×1.0	1.5	79	0/10	0
9	5	3×1.0 1×3.0	1.5	82	0/9	0
6	8	7×1.0	—	17	1/6	17
10	8	6×1.0 1×3.0	—	16	0/10	0
4	8	7×1.0	1.5	79	0/4	0
10	8	6×1.0 1×3.0	1.5	77	0/10	0
13	8[1]	—	—	23	8/13	62
14	8[1]	—	1.5	49	2/14	14

[1] Saline maintained.

controls, again indicating an additive, synergistic, or supplementary effect of the two drugs.

In all of these experiments there was definite protection against convulsion and death from water intoxication. There was no evidence that the increased diuresis in the D.C.A. and epinephrine treated animals was enhanced by additional D.C.A. (Table 2, Figure 3A and Figure 4B.)

(e) *Excretion at twenty-four hour intervals.* In Figures 1 to 4 we have included the per cent excretion for the 7 to 24 hour interval. It is clearly evident that by the end of 24 hours, a large percentage of the water was eliminated. This is particularly well illustrated in Figure 3, curves C and D. We had observed in our earlier experiment, not included in this series, that when smaller amounts of water were administered to adrenalectomized rats treated with D.C.A. the excre-

tion at the end of 24 hours was equal to that of normal controls, even though at seven hours there was a delayed diuresis.

DISCUSSION

Swingle, *et al.* (1937, 1941) have concluded that adrenalectomized dogs possess a greater sensitivity to tissue hydration, as well as renal failure, than normal dogs when subjected to excessive amounts of water by mouth. The delayed diuresis in the adrenalectomized rat according to Gaunt (1944) is explained in part by (1) a delayed in-

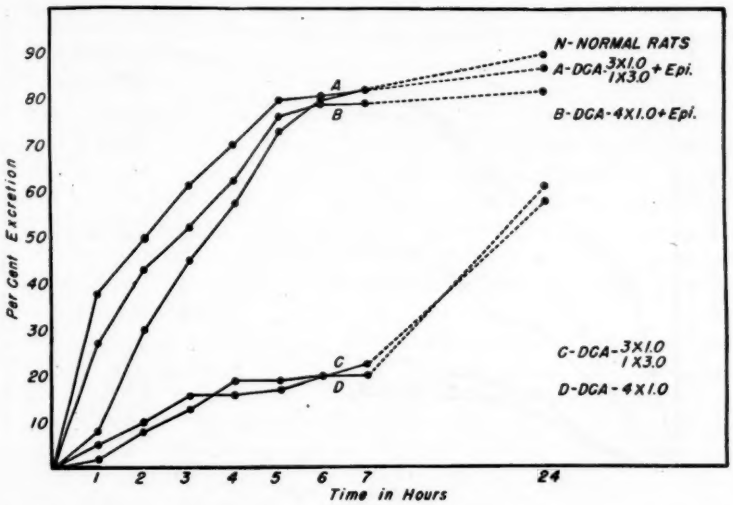

FIG. 3. Shows the effect of desoxycorticosterone acetate (curves C, D) and of D.C.A. plus epinephrine (curves A, B) on water diuresis in rats adrenalectomized 5 days. The doses of D.C.A. are expressed as days times mgs.

testinal absorption and (2) by the inability to excrete water already absorbed. Shipley (1945) contends that of the total water administered, only a small part remains in the gut, while the greatest portion is retained in the extracellular compartment, and remains unexcreted because of renal failure. It has been established also that cellular hydration (Harrop, 1936) and increased tubular reabsorption (Gersh and Grollman, 1939) may accompany adrenal insufficiency. In any event it may be assumed that the absorption was great enough to produce water intoxication.

Attempts have been made to elucidate the mechanism of delayed diuresis in adrenalectomized animals but the present concepts do not account for the difference observed in these experiments. One explanation for the restorative action of cortical extract and hypertonic sodium chloride in water intoxicated animals is the release of excess

accumulation of intracellular water, and the prevention of this accumulation of water when adrenalectomized animals are primed with extract. With D.C.A., however, the situation is somewhat different, for priming doses do not increase diuresis to normal but do prevent convulsions and death from water intoxication. Swingle *et al.* (1941) showed that D.C.A. was effective in preventing water intoxication despite the decreased diuresis. If cortical extract mobilizes and redistributes intracellular fluid by shifting electrolytes to extracellular spaces and the blood stream, it would seem reasonable that a com-

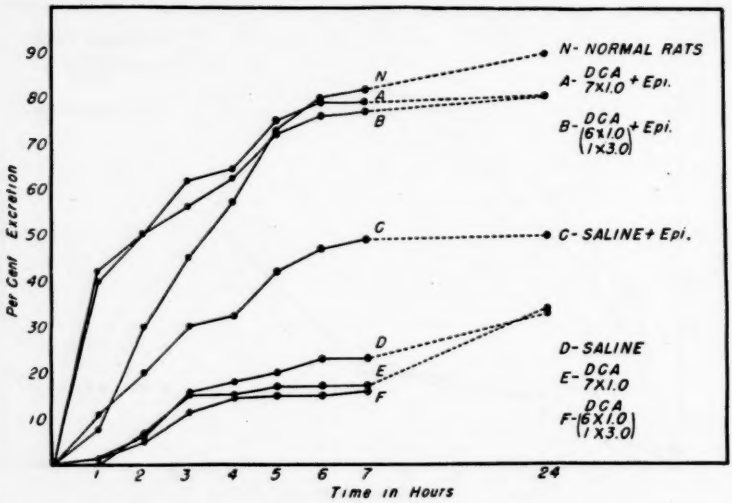

FIG. 4. Effect of D.C.A. (curves E, F); of D.C.A. plus epinephrine (curves A, B); of saline (curve D) and of saline plus epinephrine (curve C) on water diuresis in rats adrenalectomized 8 days. Doses of D.C.A. are expressed as days times mgs.

pound known to cause a retention of sodium and chloride should have some effect. What then causes the water to be eliminated in cortical-extract-primed-adrenalectomized animals and to be retained in D.C.A.-primed-adrenalectomized and normal animals? If tubular reabsorption is increased during adrenal insufficienty, then one would expect that the increased diuresis in cortical extract treated animals would be associated with a decreased tubular reabsorption. The same mechanism does not appear, however, to be involved in D.C.A. treated animals, for in these animals the marked delay in diuresis still exists but with definite protection from convulsions and death from water intoxication. It would seem thus that D.C.A. is acting in some way on the regulation of water between the various compartment.

Gaunt (1944) found that adrenalectomized rats maintained on cortical extract lose their ability to excrete water. This is also true of

adrenalectomized rats maintained on adrenal cortical transplants but to a somewhat lesser degree (Eversole, Edelman and Gaunt, 1940). It is interesting to note that Swingle, *et al.* (1941) observed no difference in convusions or diuresis between cortical extract primed adrenalectomized dogs and normals, even though the adrenalectomized dogs were previously maintained on extract for as long as four months. This difference between reported findings in dogs (Swingle) and rats (Gaunt, Hays) may be due to species differences.

The recent findings of Stein and Wertheimer (1944) that adrenal medullectomy decreases water diuresis to adrenalectomized control levels and that epinephrine restores it to normal levels suggest that the medulla plays a physiological role in water elimination were it not for the fact that recent experiments of Gaunt *et al.* (1945) show a normal response to water after demedullation. That the delayed diuresis in water intoxication is associated with the adrenal dysfunction is unquestionable but whether it is purely cortical or a combination of cortical and medullary function is debatable. Certainly it cannot be denied that epinephrine has been effective in ameliorating the symptoms of water intoxication, and the fact that adrenalectomized rats maintained on cortical extract, saline or cortical steroids become relatively refractory to water diuresis seems to indicate a more important role for the medulla.

The results of these experiments indicate that there is a marked difference in the response to water diuresis when adrenalectomized rats are treated with D.C.A. or with epinephrine in combination with D.C.A. Since in both instances there is protection from symptoms of water intoxication it is possible that with D.C.A. the distribution of water between extracellular and intracellular compartments is such as to prevent intracellular hydration without concomitant water elimination; while with epinephrine there is an elimination of the water by the kidney to prevent water intoxication. This could be explained by either (1) increased glomerular filtration or (2) decreased tubular reabsorption.

SUMMARY

Untreated adrenalectomized rats given four doses of water at hourly intervals showed a delayed diuresis and marked susceptibility to water intoxication. When treated with doses of desoxycorticosterone acetate the response to water diuresis was directly proportional to the dose, and with 2.0 mg. or more, all symptoms of water intoxication disappeared.

When rats were adrenalectomized five and eight days and maintained on 1.0 mg. D.C.A. per day, their response to water diuresis was no greater than for one-day-adrenalectomized controls, but they were protected against convulsions and death from water intoxication. Additional D.C.A. prior to the administration of water did not in any

way alter this response. Adrenalectomized rats maintained on saline showed a delayed diuresis and a mortality similar to the one-day-adrenalectomized controls.

Epinephrine HCl, when given to one-day-adrenalectomized rats, increased the diuresis from 21 to 64 per cent and decreased the mortality from 72 to 0 per cent. When 1.0 mg. D.C.A. was given in combination with epinephrine the excretion increased from 20 to 68 per cent. With 3.0 mg. D.C.A. plus epinephrine the excretion increased to 79 per cent.

Epinephrine HCl given to eight-day adrenalectomized rats maintained on saline increased the excretion from 23 to 49 per cent; while epinephrine given to D.C.A. maintained adrenalectomized rats increased the diuresis from 17 to 79 per cent. Additional D.C.A. in combination with the epinephrine did not further enhance this response. In all of these experiments there was definite protection from convulsion and death from water intoxication.

The increased diuresis with epinephrine and the protection of D.C.A. against symptoms of water intoxication in face of delayed diuresis, may be explained by either (1) an effect of epinephrine on the kidney or (2) the effect of D.C.A. on the distribution of water between extracellular and intracellular compartments to prevent excessive intracellular hydration.

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